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# CEREAL CHEMISTRY

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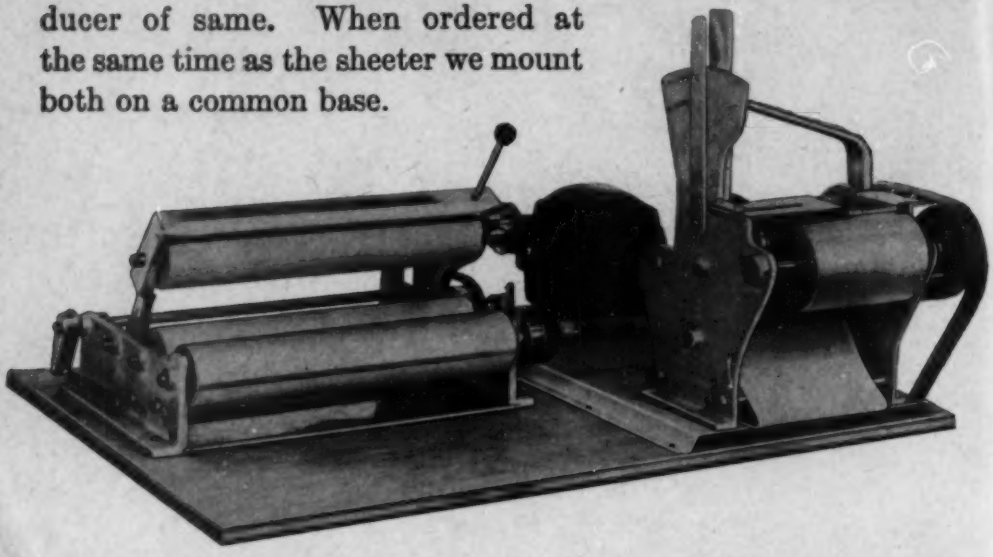

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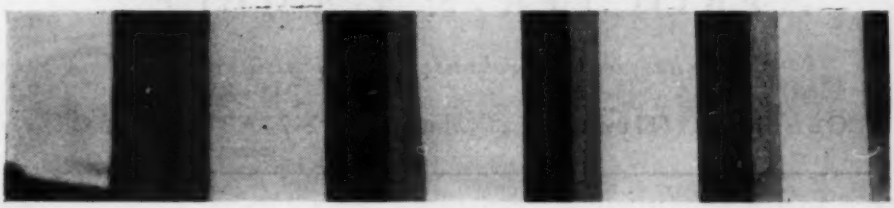

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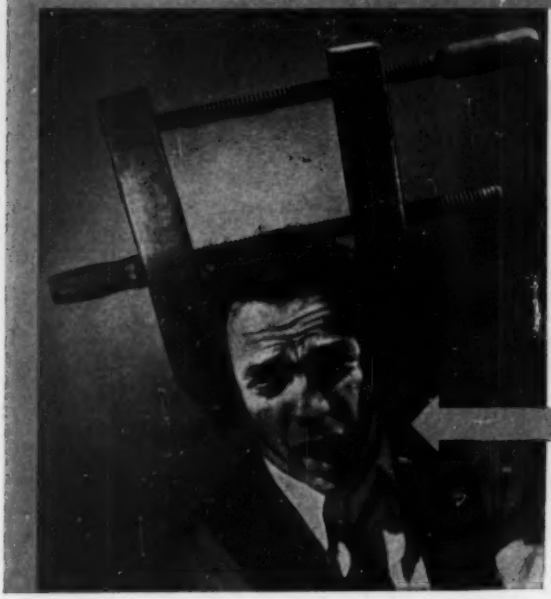

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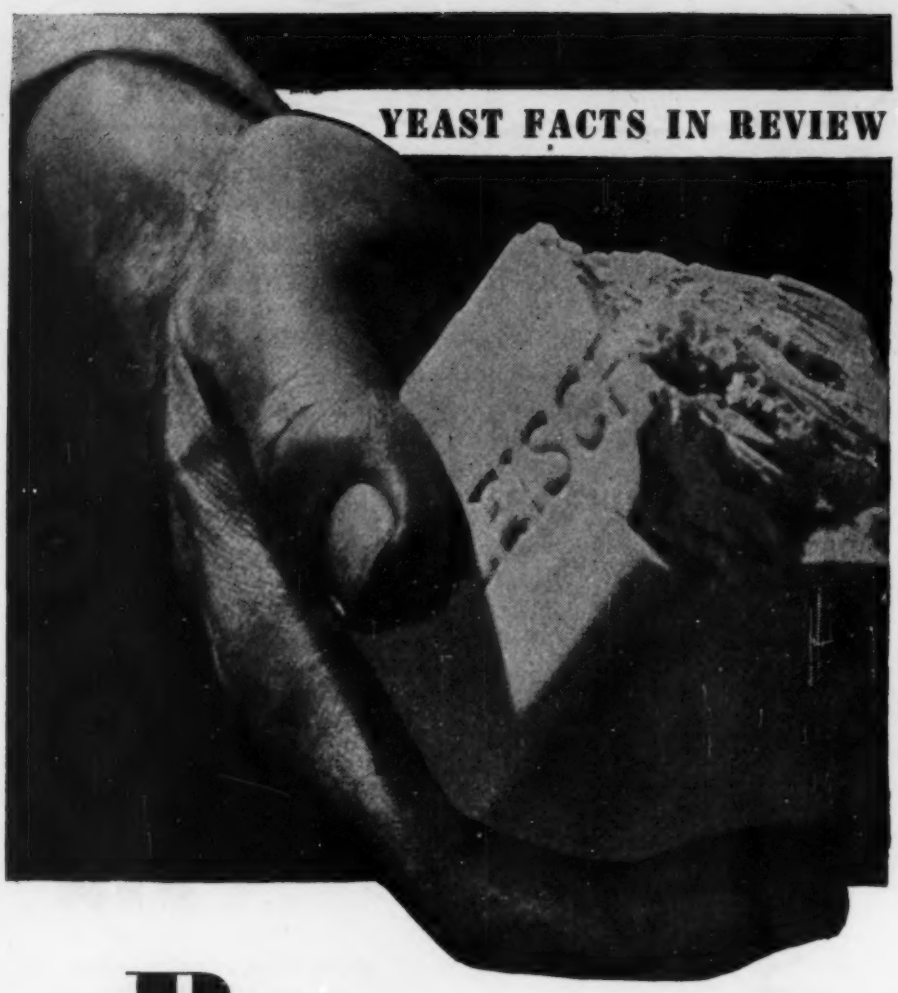


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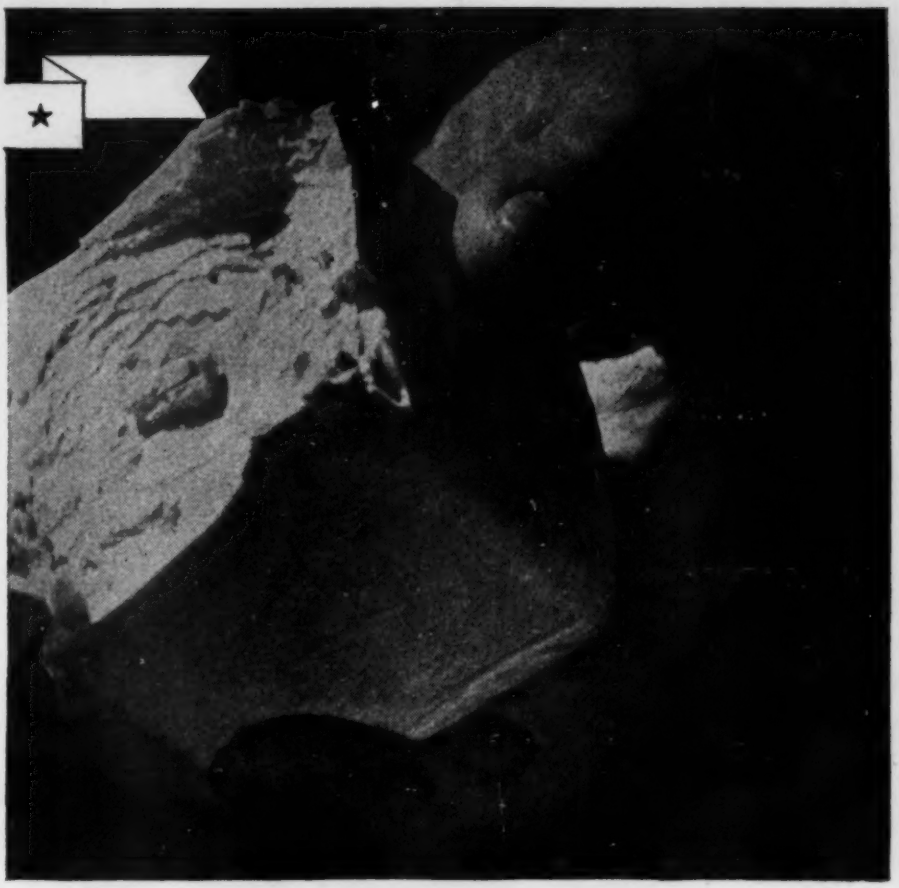

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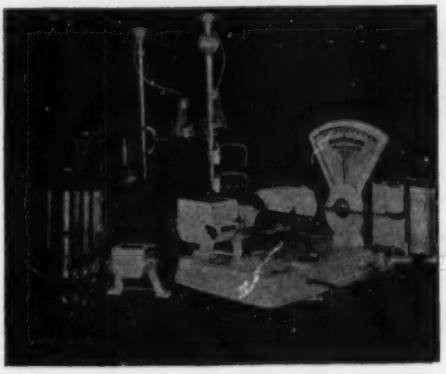

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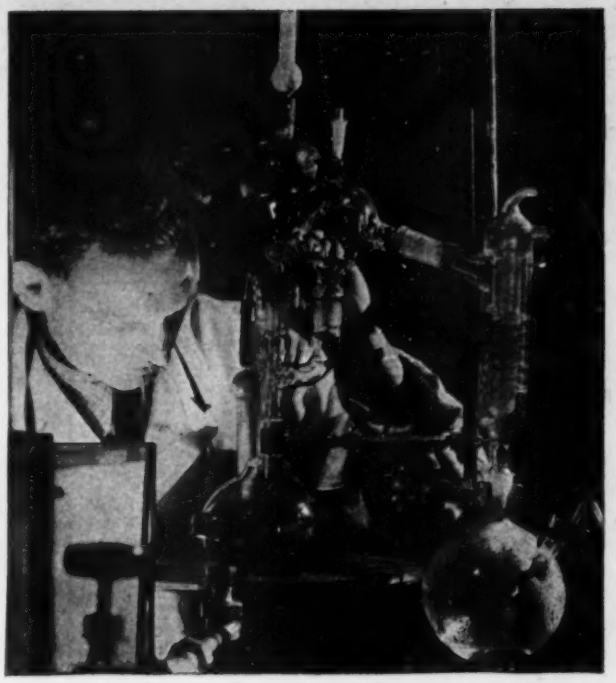

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**1938**—Nicotinic Acid Merck (Niacin) and Nicotinamide Merck (Niacinamide) were made available.

**1938**—Riboflavin Merck was the second pure crystalline vitamin to reach production during that year.

**1938**—Alpha-Tocopherol (Vitamin E) was identified and synthesized by Merck chemists and their collaborators in other laboratories.

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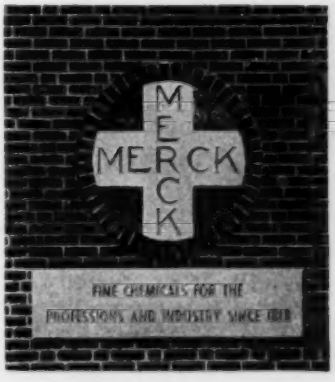

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CEREAL CHEMISTRY

VOL. XXII JULY, 1945 No. 4

# MEASUREMENT OF THE GROWTH-PROMOTING QUALITY OF DIETARY PROTEIN[1]

RICHARD H. BARNES,[2] JEAN E. MAACK, MARY J. KNIGHTS, and GEORGE O. BURR

Division of Physiological Chemistry, Department of Physiology, and the Hormel Institute. University of Minnesota, Minneapolis, Minnesota

(Received for publication January 25, 1945)

In a summary of the facts established before 1915 about the nutritive value of proteins, Osborne and Mendel (1915, 1915a) point out that certain amino acids are of dominant importance; that if a protein is relatively poor in one of these amino acids, this deficiency will become manifest if the protein intake is kept low; and, as the protein content of the diet is reduced, the intake of the limiting amino acid will be insufficient to satisfy the requirement first for growth and then for maintenance. In 1915 they undertook a study of methods of measuring quantitatively the comparative nutritive value of proteins. In a series of papers (1915, 1915a, 1916, 1919) these authors and their co-workers employed the following methods successively, each being considered an improvement over the preceding one.

(1) The basal diet, containing decreasing percentages of the test protein, was fed *ad libitum*. Food intake was measured but not controlled. Relative efficiencies for growth were found by inspection of the slopes of the growth curves on diets of equal protein content. Relative efficiencies for maintenance were found by comparing the percentage of protein in the diets on which there was no gain or loss in weight.

(2) The basal diets contained not quite enough protein to permit normal growth with *ad libitum* feeding. Weighed amounts of food were then offered each day in proportion to body size. Protein level was varied simultaneously in order to maintain essentially equal size among groups. The amounts of protein required to produce the same body gain were compared.

[1] Preliminary reports of these data were presented before the American Association of Cereal Chemists, Minneapolis, May 25, 1944, and the American Chemical Society, New York, September 11, 1944.

[2] Present address: Sharp & Dohme, Inc., Glenolden, Pennsylvania.

(3) Each protein to be tested was fed, *ad libitum*, at several levels in the basal diet. Gain in body weight per gram of protein eaten was calculated. This value is now known as the *protein efficiency*. For each protein there was an optimum level at which maximum efficiency of protein utilization for growth took place.

The authors point out that by feeding proteins at a single level, or by restricting food intake arbitrarily, only relative values can be obtained, depending upon the degree of restriction or the percentage of protein in the diet. They feel that only the third method can yield an absolute value in terms of the maximum possible efficiency of a protein. They state (1919) that the ideal conditions for the comparison of proteins would be that animals of the same starting size "eat the same amount of food in the same number of days and gain the same amount of weight, the protein factor being the only variable." As it is exceedingly difficult to conduct experiments which fulfill these requirements, they developed the procedure for finding the maximum efficiency. Their chief argument against arbitrary restriction of food is summarized in the statement: "When growth is limited by food intake, protein can be used as a source of energy and consequently a smaller part may be available for growth than when sufficient energy is supplied in other forms and growth is determined solely by protein."

The term *biological value* was originally defined by Karl Thomas (1909) as the percentage of absorbed nitrogen which was retained by the body for repair or construction of nitrogenous tissue. This measurement requires that the nitrogen intake, the nitrogen excretion, and the endogenous metabolic nitrogen be known. In 1924 Mitchell (1924) applied the method to growing rats. Later, Mitchell and Beadles (1930) emphasized the importance of paired feeding and described the methods in detail. Mitchell has critically reviewed the methods in use for determining the nutritive value of proteins (1924a, 1943, 1944). Some of his comments that bear directly upon the present investigation are the following. Referring to the work of Osborne and Mendel he says, "As originally presented the method involved a comparison of different proteins based on the *maximum* gains per gram of protein consumed. As ordinarily used by other investigators, however, no systematic attempt is made to find the maximum value; in some cases comparisons are made at one level of intake only, and in some cases comparisons of different proteins are made at different levels of intake, quite arbitrarily chosen. The values obtained with different proteins apparently stand in quite different ratios to one another, depending upon the level of protein intake." (1924a, p. 44.) And again, "The method of measuring protein quality by an efficiency ratio of growth to protein eaten implies that the protein content of the gains in body

weight of growing animals is constant regardless of the age or size of the animal, the quality of the protein or the rate of growth. . . . There is a distinct tendency for the more rapid gains in body weight to have the greater content of fat and the smaller content of protein. . . . In brief, this method of assaying food protein biologically may be expected to exaggerate quality differences among proteins when these are considerable, and to obscure them when they are inconsiderable, on account of the large experimental error to which the method is subject as it is ordinarily employed." (1944, pp. 697–698.) And finally in discussing the level of protein intake he says, "For the purpose of measuring the biological value of dietary nitrogen in promoting growth, the ideal level would seem to be that that just promotes maximum nitrogen retention. . . . *Ad libitum* feeding has no place in a protein nutrition experiment designed to obtain quantitative data." (1943, pp. 269, 276.) These last two statements do not seem to be in complete harmony since each protein may have its own optimum concentration and it can exert its maximum growth effect only if adequate nonprotein calories are available. Since this would not be attained by paired feeding of proteins at a single level it is evident that such a procedure is a compromise. It is not even clear whether pairs of animals should be held to equal food consumption or to equal gain in weight. The former is more commonly practiced. Mitchell (1924b) demonstrated that the *biological value* of several proteins is markedly affected by raising the protein content of the diet from 5% to 10%.

The main objective of the present investigation was a study of the following questions: (1) Is the measurement of body weight gain an accurate index of body protein gain? (2) What are the effects of level of protein included in the test diet? And (3) is it necessary or desirable to equalize food and protein intake by paired-feeding?

To answer these questions three experiments were done. In Experiment I the rats were pair-fed for a 54-day period and then fed *ad libitum* so that they served as their own controls. In Experiment II four groups of rats were restricted in their food consumption by those receiving the poorest of four test proteins. Experiment III was a comprehensive study of the four proteins used in II, fed *ad libitum* to 28 groups of rats, at seven levels of each protein.

## Experimental

*Experiment I.* Four groups of eight female rats (Sprague-Dawley) were put on the four experimental diets when they weighed 45 to 55 g. For 54 days a typical paired-feeding experiment was conducted in which the food intake of each rat on the better protein was restricted to that of its pair-mate on the poorer protein. At the end of this

period *ad libitum* feeding was begun. This had no effect on the poorer protein groups because they were already eating to the limit of their appetites. But the restricted pair-mates receiving the better protein immediately increased their food consumption and growth rate.

The diets consisted of sucrose, lard, salt mixture (4%), synthetic B vitamins in excess, and one drop each week of a concentrate of vitamins A, D, and E. The protein sources were mixed into diets in the amount required to give the desired level of protein and the sucrose and lard adjusted accordingly to equalize the fat content and hence the caloric value of the paired diets. With groups 1 and 2 a whole wheat flour was compared with a heat-treated wheat with distinctly poorer protein quality. Each diet contained 11.2% protein (dry basis). With groups 3 and 4 a ground white rice was compared with a heat-treated rice with distinctly poorer protein quality.

TABLE I

COMPARATIVE RESULTS WITH PAIRED AND AD LIBITUM FEEDING WHEN THE POORER DIET IS VERY RESTRICTING

(The whole wheat diet contained 11.2% protein and the white rice diet 5.65% protein expressed on a dry matter basis)

| | Average food intake | Average gain in weight | Average protein efficiency weight gain per gram protein | Ratio of P.E. by ad libitum to P.E. by restricted feeding |
|---|---|---|---|---|
| | *g/day* | *g/day* | | |
| Whole wheat diet restricted by paired feeding | 3.44 | 0.157 | 0.42 | 2.43 |
| Whole wheat diet following release to ad libitum feeding | 7.46 | 0.811 | 1.03 | |
| White rice diet restricted by paired feeding | 4.77 | 0.228 | 0.92 | 1.87 |
| White rice diet following release to ad libitum feeding | 7.03 | 0.711 | 1.72 | |

The results are summarized in Table I in which only the data for better proteins are included to show the marked effect of change from *restricted* to *ad libitum* feeding. It is clear that paired-feeding as practiced by many gives an entirely artificial value for the better protein. If the restriction is severe, some of the better protein is *wasted* as fuel for which sucrose or lard could have served as well.

*Experiment II.* Four protein sources were selected that were known to cover a wide range of nutritive values. These were petroleum ether-extracted spray-dried whole eggs,[3] a well heat-treated low-fat soyflour,[4] a poorly heat-treated low-fat soyflour,[5] and a wheat

gluten.[6] The protein sources were incorporated by replacement of sucrose and fat in the basal diet (Table II) so that the four experimental diets contained approximately 10% protein and were isocaloric.

Male albino rats (Sprague-Dawley) of weaning age were placed in individual cages and fed a commercial diet (Purina Fox Chow) for three days. At the end of the pretest period the rats were distributed according to weight among the four dietary groups of eight. The principle of paired-feeding was applied to groups of four, all being

TABLE II

COMPOSITION OF THE BASAL DIET

(Protein sources that were incorporated in the diet replaced carbohydrate and fat so that the diets remained iso-caloric)

| | |
|---|---|
| Sucrose | Varied inversely with the protein added |
| Fat [1] | 15% |
| Salt mixture [2] | 4% |
| Fiber [3] | 2% |
| B vitamins mixture [4] | + |
| Vitamins A and D [5] | + |

[1] Soybean oil was added to make a total of 15%.
[2] Wesson (1932) modification of the Osborne-Mendel salts with a trace of $ZnSO_4$ added.
[3] Cellu flour.
[4] Vitamin mixture per 100 g of diet contained:

| | |
|---|---|
| 400 μg | riboflavin |
| 400 μg | thiamine |
| 400 μg | pyridoxine HCl |
| 2,500 μg | niacin |
| 1,100 μg | calcium pantothenate |
| 7,500 μg | para amino benzoic acid |
| 200,000 μg | choline HCl |
| 20,000 μg | inositol |
| 50 μg | 2 methyl 1,4 naphthoquinine |
| 2,500 μg | α tocopherol |

The tocopherol was added to the fat and the water-soluble components were incorporated in the sucrose.
[5] Cod liver oil was administered by mouth with an eyedropper twice weekly.

limited to the food intake of the poorest eater, in this case always the rat receiving wheat gluten.

A control group of the same average weight was sacrificed at this time for determination of the initial carcass protein. The average value per unit body weight was subtracted from all final carcass protein values to determine the protein gain on each diet.

Body weight and food consumption, with special care in the recovery of spilled food, were followed for 42 days, when the rats were killed with ether, the contents of the stomach and large intestine washed out

[2] Spray-dried whole eggs extracted three times with cold petroleum ether. The protein content (N × 6.25) was 76%.
[4] Soyflour kindly supplied by Dr. J. Hayward, Archer-Daniels-Midland Co., Minneapolis, Minn. The analysis was protein (N × 6.25) 48.9% and fat 0.3%.
[5] Soyflour kindly supplied by Dr. J. Hayward, Archer-Daniels-Midland Co., Minneapolis, Minn. The analysis was protein (N × 6.25) 49.3% and fat 0.3%.
[6] Wheat gluten purchased from the Huron Milling Co., Harbor Beach, Michigan, analyzed to contain 75% protein (N × 6.25).

with water, and Kjeldahl nitrogen determinations made on the whole carcasses.

Feces were collected for four-day periods during the second, fourth, and sixth weeks of the experiment. From the nitrogen determinations apparent digestibility of each protein was calculated. These values were applied in the calculation of the percent utilization of the absorbed protein.

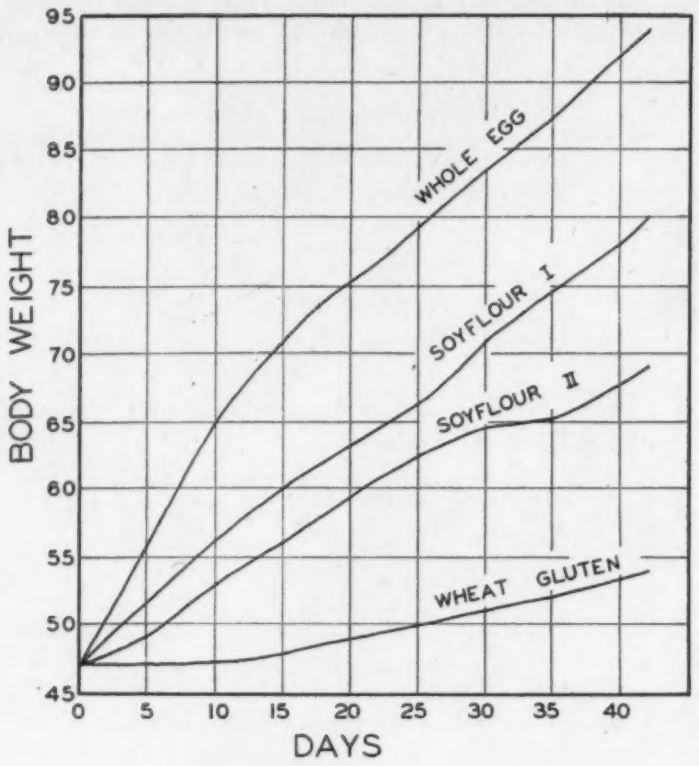


Fig. 1. Growth curves of rats that were pair-fed four proteins incorporated at a level of 10% in the diets (Experiment II).

The results of this experiment are recorded in Figure 1 as growth curves; and, along with data from Experiment III, in Figure 4 and Tables III, IV, V, and VI. In each case the data from this experiment are identified by the term "10% protein pair-fed." These results will be discussed in conjunction with those of Experiment III (below).

*Experiment III.* The materials and methods used were the same as described under Experiment II, except that *ad libitum* feeding was substituted for *paired-feeding*. Each protein was fed at the seven levels indicated in Figure 2 and Tables III and IV. The basal diets

## TABLE III

PERCENT PROTEIN (N × 6.25) IN RAT CARCASSES (MINUS INTESTINAL CONTENTS) AFTER RECEIVING EXPERIMENTAL DIETS FOR 42 DAYS

| Protein in diet (approximate) | Whole egg | Soyflour No. 1 | Soyflour No. 2 | Wheat gluten |
|---|---|---|---|---|
| % | % | % | % | % |
| | | AD LIBITUM | | |
| 4 | 15.8 | — | — | — |
| 6 | 16.2 | 17.6 | 17.9 | — |
| 8 | 16.3 | 17.8 | 18.2 | 17.1 |
| 10 | **18.0** | 17.5 | 18.0 | 17.3 |
| 12 | — | **17.6** | 18.2 | 17.5 |
| 14 | 17.6 | 18.0 | **17.7** | 17.2 |
| 18 | — | 18.4 | 19.1 | — |
| 20 | 18.2 | — | — | **16.5*** |
| 30 | — | 18.6 | 19.6 | **17.3** |
| 40 | 18.2 | — | — | **17.7** |
| | | PAIR-FED | | |
| 10 | 19.8 | 18.4 | 18.8 | **17.9** |

NOTE: Numbers in heavy type are at maximal protein efficiencies.
* This value is apparently in error.

## TABLE IV

AVERAGE PERCENT DIGESTED PROTEIN (APPARENT) FOR RATS DURING THE 42-DAY EXPERIMENTAL FEEDING PERIOD

| Protein in diet (approximate) | Whole egg | Soyflour No. 1 | Soyflour No. 2 | Wheat gluten |
|---|---|---|---|---|
| % | % | % | % | % |
| | | AD LIBITUM | | |
| 4 | 85 | — | — | — |
| 6 | 89 | 85 | 78 | — |
| 8 | 91 | 85 | 82 | 95 |
| 10 | **90** | 86 | 82 | 95 |
| 12 | — | **87** | 84 | 95 |
| 14 | 90 | 87 | **83** | 95 |
| 18 | — | 87 | 84 | — |
| 20 | 91 | — | — | **95** |
| 30 | — | 86 | 84 | **96** |
| 40 | 93 | — | — | **96** |
| | | PAIR-FED | | |
| 10 | 97 | 96 | 95 | **98** |

NOTE: Numbers in heavy type are at maximal protein efficiencies.

TABLE V

EFFECT OF EQUALIZING THE PROTEIN INTAKE ON PROTEIN EFFICIENCY VALUES

| Protein source | Maximum P.E. ad libitum feeding | P.E. at 10% protein ad libitum feeding | P.E. at 10% protein pair-fed |
|---|---|---|---|
| Wheat gluten | 0.8 | 0.3 | 0.4 |
| Soyflour No. 2 | 1.8 | 1.5 | 1.2 |
| Soyflour No. 1 | 2.1 | 2.0 | 1.8 |
| Whole egg | 3.8 | 3.8 | 2.6 |

TABLE VI

COMPARISON OF METHODS OF EXPRESSING THE GROWTH-PROMOTING QUALITY OF PROTEINS

(Whole egg protein arbitrarily set at 100 and other proteins assigned relative values)

| Protein source | Nutritive value ratios (see footnotes) | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| Whole egg | 100 | 100 | 100 | 100 | 100 | 100 |
| Soyflour No. 1 | 69 | 53 | 55 | 59 | 56 | 57 |
| Soyflour No. 2 | 46 | 40 | 47 | 48 | 55 | 55 |
| Wheat gluten | 15 | 8 | 21 | 20 | 16 | 19 |

Column 1: Grams gain in body weight per gram of protein consumed; 10% protein, pair-fed.
Column 2: Grams gain in body weight per gram of protein consumed; 10% protein, fed *ad libitum*.
Column 3: Grams gain in body weight per gram of protein consumed. Maximal ratios by *ad libitum* feeding.
Column 4: Grams gain in carcass protein per gram of protein absorbed; 10% protein pair-fed.
Column 5: Grams gain in carcass protein per gram of protein absorbed. Ratios established with 38 g of protein absorbed per rat in 42 days by *ad libitum* feeding.
Column 6: Grams gain in carcass protein per gram of protein absorbed. Maximal ratios by *ad libitum* feeding.

were iso-caloric and complete in all known vitamins, with the exception of a need for added biotin in the case of the diets containing dried egg protein. An indication of biotin deficiency was apparent on the thirty-first day. Four drops of biotin concentrate (50 μg/ml) were given by mouth and a definite increase in growth rate followed immediately. Another unexpected observation was the lack of increased growth rate of rats fed the higher levels of the poorly heat-treated soyflour (No. 2). The data in Table IV show that this is not due to exceptionally poor digestibility of the high protein diets.

The results of this experiment are summarized in Figures 2, 3, and 4 and Tables III, IV, V, and VI. The discussion will be based on the three experiments.

## Discussion

The results of these three experiments make possible at least a partial answer to the questions asked in the introduction of this paper.

(1) Is the measurement of body weight gain an accurate index of

body protein gain? The answer is to be found in Table III. With groups of eight animals, the maximum range to be expected in the average protein content is about ±11% of the mean (17.81) of all determinations. This is well within the experimental error of most biological assay methods with small numbers of animals. However, if

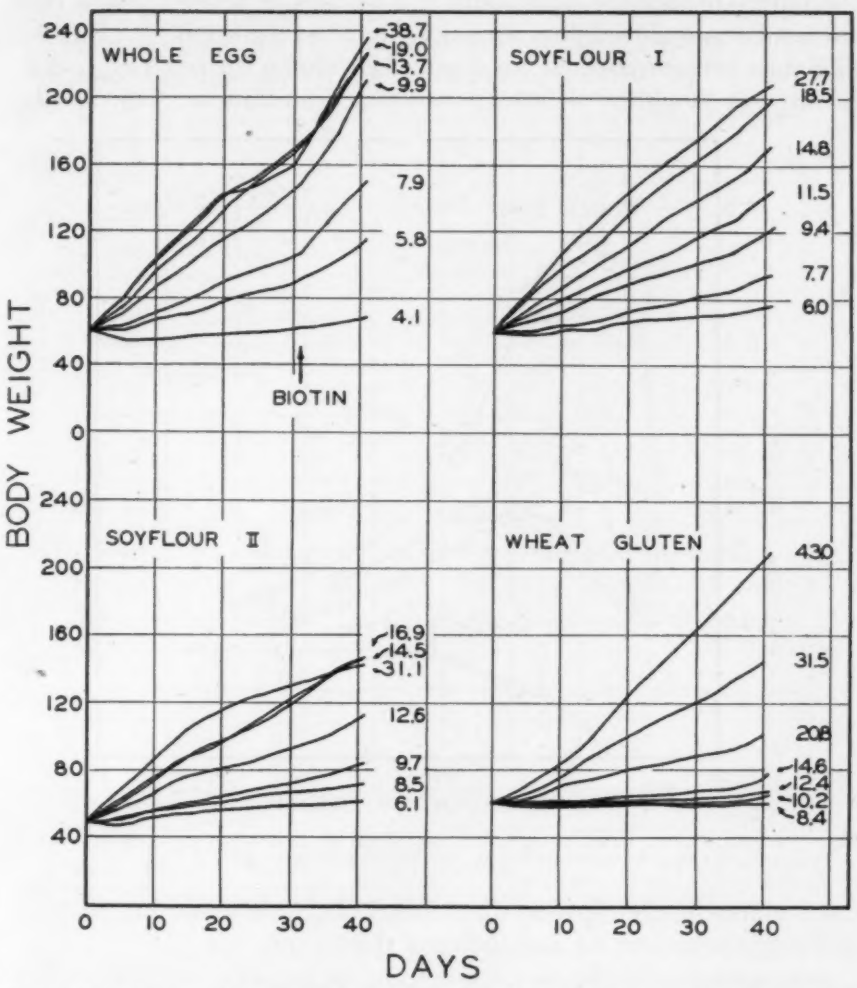


Fig. 2. Growth curves of rats consuming the indicated proteins *ad libitum*. Numbers at the right of the growth curves give the percent protein included in the diets (Experiment III).

laboratory facilities are available, nitrogen determinations will eliminate this variable. Paired-feeding does not produce a more uniform body protein. In fact, *ad libitum* feeding at the 10% level produced much more uniform animals with a maximum range of 0.7% protein as contrasted with a range of 1.9% in the pair-fed group. This same

high degree of uniformity is also found in the groups at optimal efficiency intakes (figures of Table III in heavy type). Hence with these methods of feeding, the maximum variation is probably less than other experimental errors.

A more serious objection to the use of protein efficiencies may be in the failure to measure digestibility. The data in Table IV show that the level of protein fed does not markedly affect digestibility. But the difference between soyflour No. 2 and wheat gluten is about 12%, a difference that should be included in any exact calculation. Pair-feeding

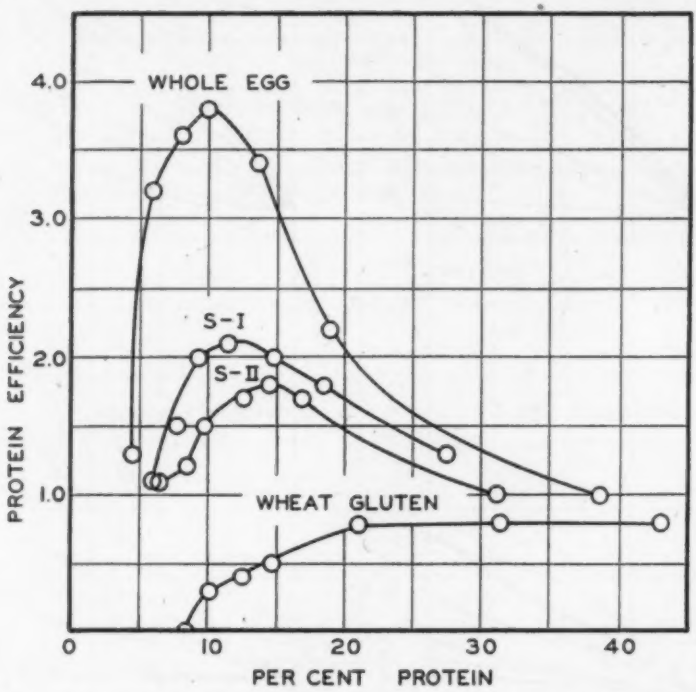


Fig. 3. Protein efficiency ratios (grams gain in body weight per gram of protein consumed) for rats ingesting four different proteins, *ad libitum* (Experiment III).

raises markedly the apparent digestibility of the higher quality proteins and tends to reduce the variability of this factor.

(2) Some of the effects of level of protein included in the test diets are shown graphically in Figures 2, 3, and 4, from which certain numerical values are tabulated in Tables V and VI. As growth rates increase on diets of higher protein content (Figure 2), the protein efficiency (Figure 3) reaches a maximum and then falls with all but the poorest protein (wheat gluten). As pointed out by Osborne, Mendel, and Ferry (1919) this optimum value is obtained with a different dietary percentage of each protein, and there is no single level of

protein at which a fair comparison of nutritive value can be made by this simple expression. Their conclusion that the maximal efficiency ratio is the most logical measure of nutritive value has been contested by some and it is apparent that investigators who propose to use this method and then arbitrarily feed the test protein at one, or, at the most, two levels may obtain results that deviate markedly from those to be obtained by a rigid application of the stipulated conditions. Although the level of protein in diets employed by different labora-

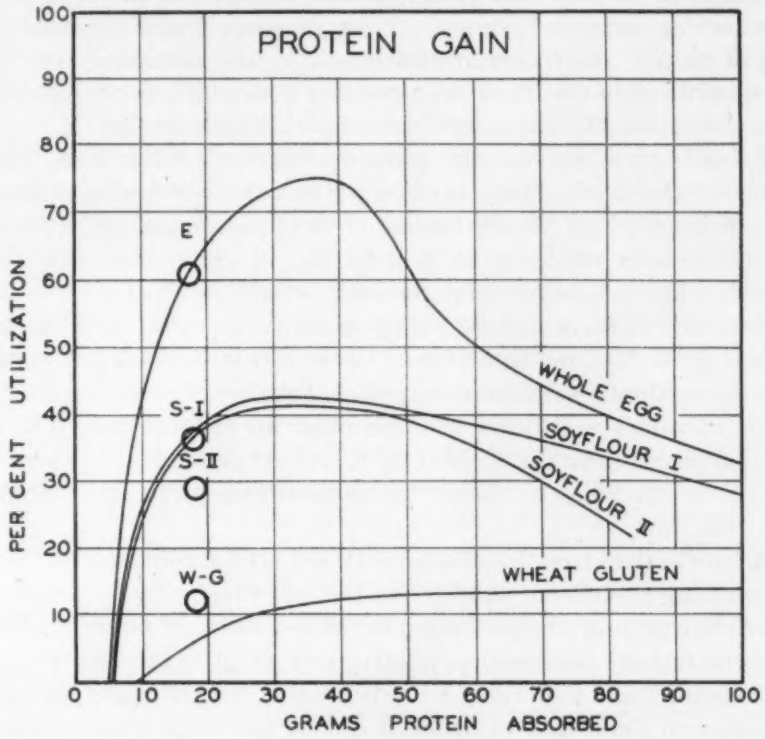


Fig. 4. The curves show the percentage of apparently absorbed protein that is utilized in the synthesis of body protein (grams gain in body protein × 100/g protein apparently absorbed) (Experiment III). The large circles represent the utilization by pair-fed rats (Experiment II).

tories may vary between 5 and 20%, that most commonly used is 10%. For proteins having an efficiency of 2.0 or greater it would appear from Figure 3 that 10% in the diet is close to optimal content. Poorer proteins, however, must be fed at considerably higher levels if abnormally low values are to be avoided.

An inspection of Figures 2 and 3 shows that there is no single growth rate which results in a maximum efficiency ratio. But there is a rate of intake of protein which gives maximum percent utilization for growth with all four proteins (Figure 4). When plotted in this

manner it is clear that maximal utilization of these four proteins occurs when about 0.75 to 1.00 g of each is absorbed each day (32 to 42 g in 42 days). Since this series of proteins represents an extreme range of quality this would appear to be a figure of some significance in determining the maximum efficiency. The curves are rather flat at the maxima and it would not be necessary to fix the protein intake at a very precise value.

For comparison with the *ad libitum* feeding curves the percentages of utilization of these four proteins, when pair-fed at the 10% level, are shown as circles in Figure 4. These illustrate graphically that the level of protein absorption arbitrarily set by the method results in a protein utilization that is on the ascending slope and is not comparable to the maximum that may be obtained with higher intakes.

Table V gives the absolute protein efficiencies obtained by three methods: (1) maximum protein efficiency by *ad libitum* feeding; (2) protein efficiency by *ad libitum* feeding of diets containing 10% protein; and (3) protein efficiency by pair-feeding of diets containing 10% protein. The maximum value for each protein results from the first method. Since by chance 10% is optimum for egg protein, the second method gives the same figure for it, but results in increasing deviation from the maximum with decreasing protein quality. The third method results in values well below the maximum for all proteins. The restriction placed upon the egg diet by the wheat gluten diet has lowered its protein efficiency to almost the same degree as in the examples shown in Table I.

All of the data from Experiments II and III are compared in Table VI, with egg protein set at 100. In the first three columns are given the relative protein efficiencies and in the last three columns data from the same rats are expressed as relative percent utilization for growth.

Columns 5 and 6 of Table VI indicate that the criteria of Osborne, Mendel, and Ferry and of Mitchell can be met by a single group of animals. If in a preliminary period the protein level of each diet is adjusted so that about 0.9 g of protein is retained each day, the nutritive value of each protein will be close to its maximum. From the data in column 3 it is evident that if facilities are not available for measuring protein balance, the simpler procedure of measuring food consumption and body weight at several levels of protein will give the same relative values for all except the protein of soyflour No. 2.

An inspection of the results obtained by each of the other procedures shows that none of them agree very well with the ideal value, but since it is not possible to test all feeds at the optimum level because of their low protein content it is well to consider the relative merits of the

substitute procedures. Protein efficiency calculated from the results of pair-feeding at the 10% level (column 1) greatly exaggerates the differences between the two soyflours, and their values with respect to egg protein are out of line. This fault is partially corrected by calculations of protein retention (column 4). Protein efficiency calculated from the results of *ad libitum* feeding at the 10% level (column 2) tends to penalize the poorer proteins, each of which has a lower value relative to egg protein than is given by any other procedure. This exaggeration of differences between good and poor proteins has been pointed out by Mitchell (1944).

However, the good *relative* agreement obtained by pair-feeding at the 10% level (column 4) is likely to give workers the erroneous view that results are essentially as good as the maximal ratios (column 6). This is not the case because they are all relative and none of them have any absolute value. In Mitchell's work (1924b) the biological value increased as much as 13% when the protein level was dropped from 10% to 5% of the diet. In one case this reversed the relative ranks of the proteins. At the 5% level potato protein ranked below corn and oats, while at the 10% level it was superior to both. His results emphasize the findings here that the absolute efficiency given by any arbitrary percentage of dietary protein is a matter of chance, depending upon the several factors discussed.

Finally, if the simple protein efficiency calculations are to be made from an experiment with diets containing 10% protein, pair-feeding will give the better relative values to the very poor proteins (Table VI), but *ad libitum* feeding gives the better absolute values for all but the poorest protein (Table V), whose value is independent of the feeding method since the animals eat all they want in either case.

## Summary

A study was made of the influence of the level of protein consumption upon the utilization of proteins in growth of rats.

The maximal utilization of absorbed protein for the synthesis of body protein has been established for four crude protein sources differing widely in nutritive quality. The amount of each protein absorbed when maximal growth utilization was attained was found to be essentially a constant.

From theoretical considerations, the maximal utilization of absorbed protein for the synthesis of body protein is the most valid expression of the growth-promoting quality of dietary protein.

The establishment of the maximal ratio of body weight gained to protein consumed is the most useful of the methods considered here, of measuring nutritive value of proteins for growth, that do not involve

fecal and carcass nitrogen analyses, but it does not necessarily provide wholly accurate indices of protein values.

The common practice of measuring gain in body weight as a function of protein consumption with diets fed *ad libitum* and containing an arbitrarily selected amount of protein may be useful in a preliminary rating of proteins, but should not be given wide application without a full understanding of its limitations.

Restricting the amount of proteins ingested by the paired-feeding of two or more proteins of widely differing nutritive values results in relative efficiency values which vary with the percent of protein in the diet to such a degree that relative ranks may be changed. The absolute values obtained by this method at the 10% level are far from the maximum when calculated either as protein efficiencies or as protein utilization for growth.

By all of the methods reported here the proteins keep the same relative rank, the degree of difference changing with method of calculation.

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# THE NUTRITIVE QUALITY OF SOME PLANT PROTEINS AND THE SUPPLEMENTAL EFFECT OF SOME PROTEIN CONCENTRATES ON PATENT FLOUR AND WHOLE WHEAT

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The nutritive value of a protein depends on the kind and amount of amino acids it supplies to the animal body. The evaluation of the comparative nutritional quality of food protein is an exceedingly complex problem. Proteins vary considerably in their digestibility and in the extent to which the end products of their digestion are absorbed from the digestive tract and utilized for body functions.

There are three approaches to the determination of protein quality: (a) the determination of the amino acid content of the food protein by either chemical or microbiological methods; (b) the determination of the ratio of gain in weight to protein intake for growing animals (often called the rat growth method); and (c) the determination of the amount of nitrogen retained by animals (usually referred to as the nitrogen balance method). All of the proposed methods for determining protein quality are subject to criticism. The attributes and limitations of these methods have been discussed in a review by Mitchell (1944).

The most commonly employed biological method for evaluating protein quality involves the determination of the ratio, usually termed protein efficiency, of gain in body weight to protein consumed when growing rats are fed a diet in which the protein is the only factor limiting growth. The method was proposed by Osborne, Mendel, and Ferry (1919). These workers demonstrated that the numerical expression for protein quality depends on the level of protein in the diet, and that a maximum protein efficiency could be obtained by varying the concentration of the protein in the test diet.

Most investigators using the rat growth method have employed only one arbitrarily selected protein level, usually the 10% level. Strictly speaking, this procedure is not correct, yet precedent for this course was set by Osborne and Mendel (1919). In a recent critical study of the factors affecting the measurement of the nutritive value of proteins, Barnes, Maack, Knights, and Burr (1945) corroborated the findings of Osborne *et al* (1919) and demonstrated that for proteins having a protein efficiency of 2.0 or greater, the maximal ratio was obtained when the dietary protein level was approximately 10%. However, the protein

efficiency value for a poor-quality protein was considerably below the maximal ratio when protein level was 10%.

Another frequent criticism of the rat growth method is that the diet is fed *ad libitum*. It is argued that protein efficiency values cannot be used as an index of protein quality unless the amount of protein consumed is the same for each protein assayed. Barnes *et al* (1944) demonstrated, however, that the protein efficiency value of a high-quality protein is decreased when the food consumption of rats fed a diet containing a high-quality protein is restricted to that of animals fed a diet containing a low-quality protein. Restricting the amount of protein ingested resulted, for the proteins tested, in a decrease in the efficiency of utilization. These investigators feel that *ad libitum* feeding gives a more accurate measure of protein quality than does the paired-feeding technique.

Although the rat growth method employing a single dietary protein level may not give an absolute measure of the quality of various proteins, it yields useful information since the practical test of any feed or food combination is generally measured by its ability to produce good growth. The method measures the value of proteins for growth but does not assess their utilizability for maintenance, pregnancy, or lactation. The growth period is the most critical stage of an animal's life and it can be assumed that if a protein supplies an adequate amount of each of the amino acids required for growth it will also be a good source of amino acids for other body functions.

The nutritive value of the proteins in whole wheat and its milling fractions have been studied using the rat growth method by Osborne and Mendel (1919, 1920), Hoagland and Snider (1927), and Chick (1942). All of these workers either used a yeast supplement to supply the B-complex vitamins or assumed that the material under test supplied them in an adequate amount. Many of the supplements supplied as much as 15 to 25% of the total protein in the diet; undoubtedly this influenced the values obtained.

The present investigation was carried out in order to study the nutritive value of some wheat products and the supplemental value of some high-quality plant proteins to the protein of patent flour and whole wheat.

## Materials and Methods

Protein quality was determined by a rat growth procedure similar to the method reported by Osborne *et al* (1919). The basal ration contained the protein source under test in an amount calculated to furnish 10% protein. The other ingredients included 4% salt mixture,[1]

[1] Phillips and Hart (1935) salt mixture with manganese sulfate ($MnSO_4 \cdot 4H_2O$) increased from 0.7 g/kg to 10 g/kg of salt mixture.

4% corn oil, 1% liver extract powder,[2] and the balance sucrose. Synthetic B-complex vitamins were fed daily by dropper at the following levels: 10 μg thiamine, 20 μg riboflavin, 30 μg pyridoxine, 90 μg calcium pantothenate, and 8.6 mg of choline chloride. Halibut liver oil diluted 1 : 10 with corn oil was fed by dropper at a level of four drops weekly to supply vitamins A and D. The material under test and the prepared rations were analyzed for protein and moisture (Table I).

The liver extract powder at 1% level furnished 0.5% protein, bringing the total protein of the ration to 10.5%. Since the protein in the liver extract powder has poor quality and probably does not have

TABLE I

ANALYSIS OF INGREDIENTS AND RATIONS

| Material | Material analysis | | Ration analysis | |
|---|---|---|---|---|
| | $N \times 6.25$ | Moisture | $N \times 6.25$ | Moisture |
| | % | % | % | % |
| Whole wheat | 14.4 | 12.6 | 10.7 | 7.6 |
| Wheat germ | 29.9 | 10.0 | 10.6 | 4.0 |
| Wheat bran | 16.0 | 13.0 | 10.4 | 7.5 |
| Shorts | 17.4 | 11.9 | 10.6 | 6.9 |
| Red dog | 17.7 | 11.2 | 10.7 | 6.5 |
| Second clear | 17.4 | 11.2 | 10.4 | 7.2 |
| First clear | 16.2 | 12.2 | 10.4 | 7.3 |
| Patent flour | 13.5 | 13.2 | 10.3 | 9.2 |
| Nonfat dry milk solids | 34.6 | 6.4 | 10.1 | 2.6 |
| Corn gluten meal | 47.1 | 9.8 | 10.7 | 2.5 |
| Corn oil meal | 24.0 | 9.8 | 10.1 | 4.7 |
| Cottonseed meal | 40.9 | 8.3 | 10.4 | 2.1 |
| Linseed meal | 35.5 | 10.0 | 10.5 | 2.9 |
| Soybean oil meal | 42.3 | 9.6 | 10.8 | 3.0 |
| Low-fat soy flour | 48.7 | 6.3 | 10.9 | 1.4 |
| Low-fat wheat germ | 34.8 | 9.7 | 10.2 | 12.4 |
| Peanut meal and hulls | 39.8 | 6.8 | 10.8 | 2.2 |

any significant supplementing value (Hove and Harrel, 1943), the protein intake was considered as the amount of protein supplied by the material under test. That is, total protein of the ration minus protein supplied by the liver extract powder equals the protein furnished by the test material. Protein efficiency (P.E.) was calculated by dividing the gain in body weight during a 6-week test period by the protein intake (furnished by the test material) during the same period.

Male rats of the Sprague-Dawley strain weighing 35 to 45 g were used throughout this investigation. They were housed individually in a temperature-controlled room. Food consumption and body weight records were taken weekly for a period of 6 weeks.

[2] Liver concentrate powder 1:20—the Wilson Laboratories, Chicago, Ill.

Throughout this work, protein was expressed as $N \times 6.25$. This factor is usually used when calculating the protein content of diets containing various protein sources, but it is evident that it will not give the most favorable protein efficiency values to cereal products. In the milling industry it is customary to use 5.7 as the factor for converting nitrogen to protein, whereas the factor 6.38 is used in computing the protein content of milk. The results on cereal products would be increased approximately 9%, while those of milk products would be decreased by approximately 2% if the customary factors were used.

## Results

A blend of hard spring wheats and representative samples of the milling fractions of this blend were assayed for protein quality. The

TABLE II

PROTEIN QUALITY OF WHEAT MILLING FRACTIONS

| Material | Number of rats | Protein in ration | Weight gain mean | Protein consumed mean | Protein efficiency | | |
|---|---|---|---|---|---|---|---|
| | | | | | Mean | Range | Std. error [1] |
| | | % | g | g | | | |
| Whole wheat | 10 | 10.2 | 41.9 | 30.0 | 1.40 | 1.30–1.53 | 0.09 |
| Wheat germ | 10 | 10.1 | 131.8 | 45.9 | 2.87 | 2.72–3.02 | 0.09 |
| Wheat bran | 10 | 9.9 | 101.5 | 47.3 | 2.15 | 1.96–2.26 | 0.09 |
| Shorts | 10 | 10.1 | 124.0 | 50.6 | 2.45 | 2.31–2.65 | 0.11 |
| Red dog | 10 | 10.2 | 77.7 | 37.7 | 2.06 | 1.79–2.22 | 0.12 |
| Second clear | 10 | 9.9 | 33.5 | 27.9 | 1.20 | 1.06–1.35 | 0.09 |
| First clear | 10 | 9.9 | 19.1 | 23.0 | 0.83 | 0.63–1.03 | 0.13 |
| Patent flour | 10 | 9.8 | 19.2 | 22.9 | 0.84 | 0.76–0.98 | 0.06 |

[1] Single determination.

TABLE III

PROTEIN QUALITY OF VARIOUS PROTEIN CONCENTRATES

| Material | Number of rats | Protein in ration | Weight gain mean | Protein consumed mean | Protein efficiency | | |
|---|---|---|---|---|---|---|---|
| | | | | | Mean | Range | Std. error [2] |
| | | % | g | g | | | |
| Nonfat dry milk solids | 10 | 9.6 | 125.0 | 44.1 | 2.84 | 2.48–3.20 | 0.22 |
| Corn oil meal | 9[1] | 9.6 | 106.9 | 41.7 | 2.56 | 2.42–2.71 | 0.11 |
| Corn gluten meal | 11 | 10.2 | 14.0 | 20.6 | 0.68 | 0.38–0.82 | 0.13 |
| Cottonseed meal | 11 | 9.9 | 43.5 | 33.0 | 1.32 | 1.10–1.53 | 0.15 |
| Linseed meal | 11 | 10.0 | 72.5 | 38.5 | 1.88 | 1.60–2.14 | 0.18 |
| Soybean oil meal | 11 | 10.3 | 88.6 | 41.3 | 2.14 | 1.80–2.53 | 0.19 |
| Low-fat soy flour | 11 | 10.4 | 91.5 | 40.7 | 2.25 | 1.94–2.62 | 0.20 |
| Low-fat wheat germ | 11 | 9.7 | 133.0 | 46.6 | 2.86 | 2.70–3.14 | 0.11 |
| Peanut meal and hulls | 11 | 10.3 | 48.4 | 33.1 | 1.46 | 1.24–1.69 | 0.16 |

[1] One rat died during the third week of the experiment.
[2] Single determination.

results are shown in Table II. Wheat germ has a protein efficiency value higher than that of any of the other milling fractions. It compares favorably with nonfat dry milk solids (Table III) as a source of excellent quality protein. The nutritive value of the proteins contained in shorts, bran, and red dog is approximately the same as that of soybean protein (Table III). The milling fractions containing only small amounts of germ and bran showed poor protein quality.

The protein efficiency values of various protein concentrates are given in Table III. All of these materials except the low-fat soy flour and the nonfat dry milk solids are of the "animal feed" grade. The low-fat soy flour contained approximately 7% fat. The low-fat wheat

TABLE IV

PROTEIN QUALITY OF MIXTURES OF PATENT FLOUR AND SUPPLEMENTARY PROTEIN CONCENTRATES

| Patent flour | Supplementary material | Supplemental protein of total protein | Number of rats | Protein in ration | Weight gain mean | Protein consumed mean | Protein efficiency | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Mean | Std. error [2] |
| % | % | % | | % | g | g | | |
| 90 | 10 Corn oil meal | 18 | 9 | 9.7 | 21.6 | 23.1 | 0.93 | 0.15 |
| 80 | 20 Corn oil meal | 32 | 9 | 9.9 | 50.7 | 33.4 | 1.52 | 0.18 |
| 60 | 40 Corn oil meal | 58 | 9 | 10.1 | 89.7 | 43.0 | 2.09 | 0.10 |
| 90 | 10 Soybean oil meal | 26 | 9 | 10.1 | 55.0 | 34.5 | 1.59 | 0.14 |
| 80 | 20 Soybean oil meal | 44 | 9 | 9.7 | 75.9 | 36.9 | 2.06 | 0.03 |
| 97 | 3 Nonfat dry milk solids | 7 | 9 | 9.8 | 25.0 | 24.9 | 1.00 | 0.10 |
| 94 | 6 Nonfat dry milk solids | 14 | 9 | 9.7 | 31.1 | 26.1 | 1.19 | 0.17 |
| 80 | 20 Nonfat dry milk solids | 39 | 9 | 9.7 | 51.7 | 29.2 | 1.77 | 0.03 |
| 97 | 3 Low-fat wheat germ | 7 | 9 | 9.7 | 26.1 | 23.7 | 1.10 | 0.03 |
| 94 | 6 Low-fat wheat germ | 14 | 8[1] | 9.6 | 36.9 | 27.2 | 1.36 | 0.12 |
| 80 | 20 Low-fat wheat germ | 40 | 9 | 9.8 | 71.4 | 35.9 | 1.99 | 0.11 |

[1] Data for one rat omitted.
[2] Single determination.

germ (solvent extracted) was from the same source as the wheat germ previously described. Nonfat dry milk solids, low-fat wheat germ, and corn oil meal were found to contain protein of excellent quality. Corn gluten meal contained protein of very poor quality.

The supplemental value of several protein concentrates to patent flour was determined by mixing varying amounts of the concentrates with the patent flour and calculating the protein efficiency values of the mixtures. The sources of supplementary protein were nonfat dry milk solids, low-fat wheat germ, corn oil meal, and soybean oil meal. The results of these studies are listed in Table IV, and illustrated graphically in Figure 1. The curves in Figure 1 were constructed by plotting the mean protein efficiency value against percent of supplemental protein of the total protein of the mixture. A supplemental

effect results when the protein efficiency value of a mixture of two or more proteins is larger than the predicted value for the dilution. When the protein efficiency value of a mixture is the same as the predicted value, there is no supplemental effect.

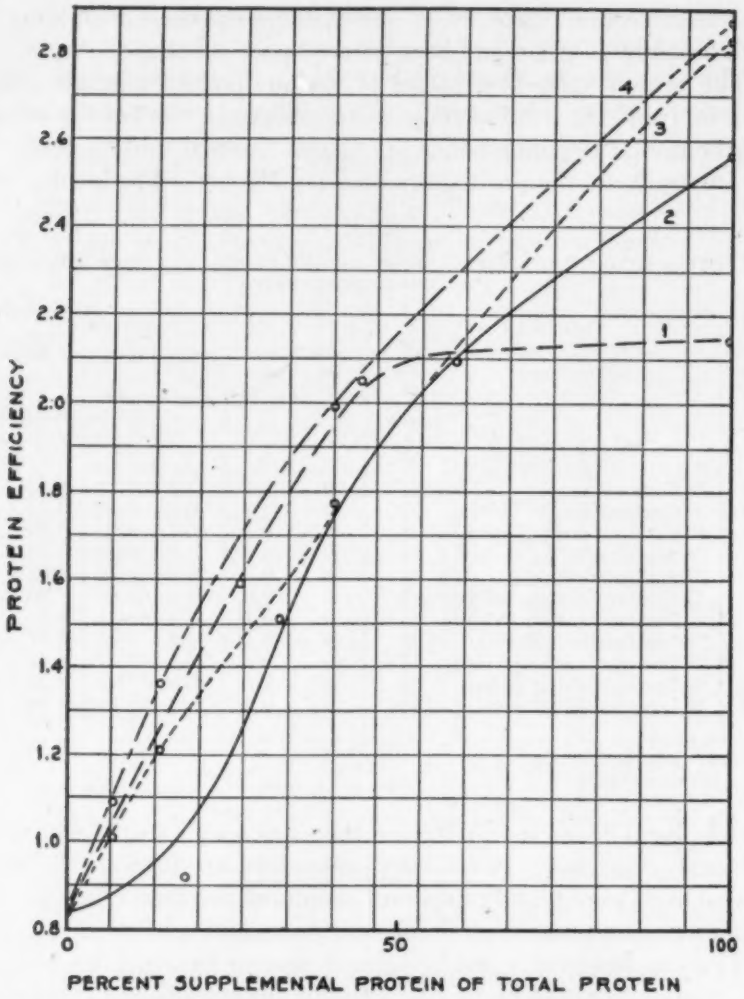


Fig. 1. Supplemental effect of some plant proteins to patent flour: (1) soybean oil meal, (2) corn oil meal, (3) Nonfat dry milk solids, (4) low-fat wheat germ.

Soybean oil meal exhibited a marked supplemental effect on the protein in patent flour. Nonfat dry milk solids, low-fat wheat germ, and corn oil meal were about equal in their supplemental value. The supplemental effect of corn oil meal did not become apparent until it supplied about 30% of the total protein.

Of particular interest is the 6% level of nonfat dry milk solids since it is common practice among bakers to use approximately this amount in making bread. The protein efficiency value of patent flour was increased from 0.84 to 1.19 by this addition. Also of interest is the 3% wheat germ level since this is roughly the amount of germ in whole wheat. This level of germ increased the protein efficiency value of patent flour to 1.10.

The supplemental value of protein concentrates to whole wheat was determined by mixing them in various proportions with the blend of tempered hard spring wheats, ground to pass a 24 mesh screen. The results of these studies are given in Table V and illustrated graphically in Figure 2.

TABLE V

PROTEIN QUALITY OF MIXTURES OF WHOLE WHEAT AND SUPPLEMENTARY PROTEIN CONCENTRATES

| Whole wheat | Supplementary material | | Supplemental protein of total protein | Number of rats | Protein in ration | Weight gain mean | Protein consumed mean | Protein efficiency | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Mean | Std. error [1] |
| % | % | | % | | % | g | g | | |
| 90 | 10 | Soybean oil meal | 25 | 9 | 10 | 66.7 | 34.3 | 1.94 | 0.12 |
| 80 | 20 | Soybean oil meal | 42 | 9 | 9.8 | 100 | 42.5 | 2.35 | 0.11 |
| 65 | 35 | Soybean oil meal | 59 | 9 | 9.7 | 101 | 41.2 | 2.45 | 0.10 |
| 50 | 50 | Soybean oil meal | 74 | 9 | 9.6 | 107 | 42.4 | 2.53 | 0.18 |
| 90 | 10 | Corn oil meal | 16 | 9 | 9.8 | 54.7 | 32.6 | 1.68 | 0.11 |
| 80 | 20 | Corn oil meal | 30 | 9 | 10 | 69.2 | 37.8 | 1.83 | 0.11 |
| 60 | 40 | Corn oil meal | 54 | 9 | 10 | 95.3 | 46.1 | 2.07 | 0.18 |
| 0 | 50 | Soybean oil meal | 100 | 9 | 9.9 | 95.1 | 40.3 | 2.36 | 0.20 |
| | 50 | Corn oil meal | | | | | | | |
| 65 | 17.5 | Soybean oil meal | 56 | 9 | 10 | 101 | 42.1 | 2.40 | 0.19 |
| | 17.5 | Corn oil meal | | | | | | | |

[1] Single determination.

Soybean oil meal showed a marked supplemental effect on whole wheat. A mutual supplemental effect was exhibited when about 50% of the total protein was supplied by soybean oil meal, indicating that such a mixture probably furnished the animal with a more adequate amino acid balance than either material alone. Although corn oil meal contains high-quality protein, it did not exert any appreciable supplemental effect on whole wheat.

## Discussion

Jones and Divine (1942), using a synthetic type ration and employing the single protein level of 9.1% in the diet (they do not state whether or not the protein factor used was 5.7 or 6.25), assayed patent

flour, whole wheat, soybean oil meal, and combinations of the meals with patent flour. The protein efficiency values for whole wheat and patent flour were 1.15 and 0.75, respectively. Chick (1942) reported protein efficiency values for whole wheat of 1.66 to 1.77 on a ration containing about 12% protein; recalculating her results to the 6.25 factor, her average value for whole wheat would be 1.5. The values reported by these workers are in good agreement with those reported herein.

Wheat is an important food and protein source, not only for humans in the form of flour and bread (close to 30% of the total protein of the

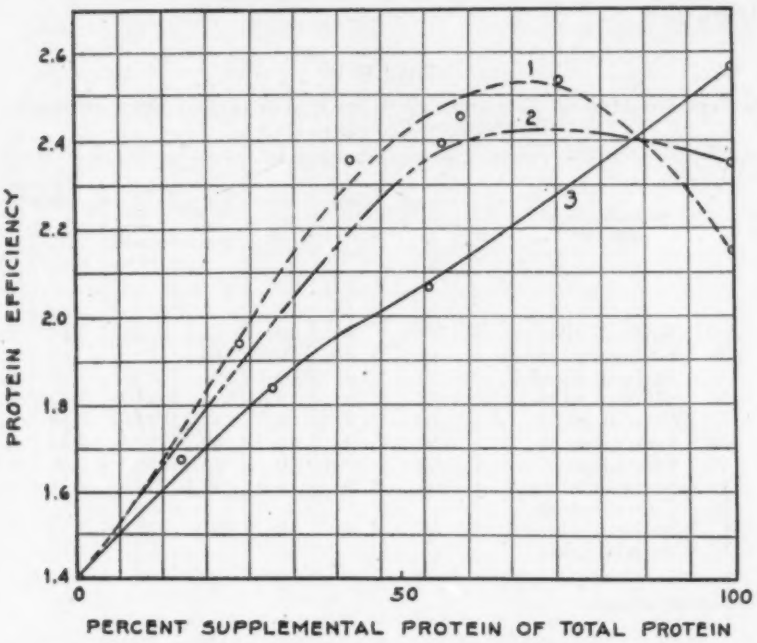


Fig. 2. Supplemental effect of some plant proteins to whole wheat: (1) soybean oil meal, (2) soybean oil meal—corn oil meal 1 : 1, (3) corn oil meal.

American diet was derived from this source in 1941) (Maynard, 1942), but also for domestic animals in the form of the by-products of the milling industry. Within the limits of the method employed, the data presented in this paper show the comparative distribution of the better quality wheat proteins among the various products of the milling process and the supplemental value of certain plant proteins to patent flour and whole wheat. In periods of shortages of good-quality animal proteins both for man and animals, such data should be useful in planning a better and more efficient utilization of the plant substances which show good protein quality.

**Summary**

Representative fractions from the milling of a single sample of commercially blended hard spring wheats were assayed for protein quality by the rat growth method with *ad libitum* feeding. The protein efficiency values ranged from 0.84 for patent flour to 2.86 for wheat germ (based on a conversion factor of 6.25).

Commercial feed samples of various plant protein concentrates were assayed for protein quality. Wheat germ, shorts, corn oil meal, bran, and soybean oil meal were found to contain high quality protein.

Patent flour was supplemented with various protein concentrates. Nonfat dry milk solids and wheat germ were about equal in their ability to improve the nutritive quality of patent flour. Six percent nonfat dry milk solids added to white flour resulted in a protein efficiency value only slightly lower than the value for whole wheat.

Whole wheat was supplemented with various protein concentrates. Soybean oil meal and whole wheat showed mutual supplementation in that mixtures possessed better nutritive quality than either component alone.

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# FUNCTIONAL PROPERTIES OF SOYA FLOUR AS A BREAD INGREDIENT

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While there are many varieties of soya beans that have been grown from time to time in various sections of the world and in certain areas of the United States, there are relatively few varieties that are grown commercially in any one particular area.

The state experiment stations have taken great interest in developing varieties of soya beans that will produce large yield and high quality beans under the soil and climatic conditions existing in their state. At the present time more than one hundred named varieties are handled by domestic growers and seed men and are under test by state and federal agencies.

The methods used in processing soya beans into flour are described by Bailey, Capen, and LeClerc (1935). Each process has its own particular method of debittering beans from which flour of fine granulation is produced by suitable pulverizing equipment.

In the last few years the production of edible grades of soya flour has increased tremendously; thus, in 1939, 25,000,000 lb of all types were produced, and in 1943, the quantity was well over a billion lb. With large quantities of edible soya flour available, it is of interest to examine the properties of various types of soya flours as they affect the production of commercial white bread.

Scientific data on the function of soya flour in yeast-raised products are very limited. In 1935, Bailey, Capen, and LeClerc reviewed the literature and reported their observations on the value of soya flour as a bread ingredient, both from the nutritional and bakery standpoint.

Increasing recognition of the valuable amino acid composition of soya flour protein when used in combination with the proteins of wheat flour and milk, as reported by Harris, Clark, and Lockhart (1944) and Hayward and Hafner (unpublished) has made it increasingly important to evaluate the action of the edible grades of soya flour as an ingredient of yeast-raised products, particularly white bread.

The types of edible soya flours produced can be classified into three groups: full fat, expeller pressed, and solvent extracted. Typical analyses of flours commercially available in each group are as follows:

TYPICAL ANALYSIS

| Type | Moisture | Ash | Fat | Protein N × 6.25 | Crude fiber | Nitrogen-free extract |
|---|---|---|---|---|---|---|
| | % | % | % | % | % | % |
| Full fat | 5.0 | 4.75 | 23.5 | 41.5 | 2.3 | 23.0 |
| Expeller pressed | 5.0 | 5.50 | 7.5 | 50.0 | 3.0 | 39.0 |
| Solvent extracted | 8.0 | 5.75 | 1.8 | 52.0 | 3.0 | 32.4 |

The soya flours selected for use in the experiments reported in this paper were those which repeated baking tests have shown were the best of their type. Specifications for the edible grades have been established by Food Distribution Administration, Grain Products Branch, which, with a few additions, can be used for outlining specifications for soya flour for use in bread and other yeast-raised products.

The addition of a thermophile specification limiting the number of such spores, some of which develop typical rope in bread, to not over 50 per gram, guarantees a satisfactory raw material for inclusion in yeast-raised goods.

## Experimental

*Rope Spore Content.* No great emphasis was placed on thermophiles in edible soya flour until the requirements of manufacturers using soya flour in bread and canned food items necessitated the production of a flour low in heat-resisting spores.

Rope spores in various brands and types of soya flour were determined by the method suggested by Hoffman, Schweitzer, and Dalby (1937) with the results shown below. Commercial experience has shown that if the rope spores in soya flour are kept below a maximum of 50 per gram, no trouble will be experienced with rope in bread under conditions normally used to control rope development.

| Brand | Spores per gram |
|---|---|
| A | 2 |
| B | 200+ |
| C | 200+ |
| D | 20 |
| E | 200+ |

*Yellow Pigment Content.* The yellow pigment content of a series of soya flours was determined by Ferrari's method (1933) using n-butyl alcohol saturated with water as the solvent, as recommended by Binnington, Sibbitt, and Geddes (1938). The transmittancy of the extract was determined in a Lumitron Colorimeter using a 440 mμ filter and 22 mm test tube cuvettes. Since no attempt was made to separate this color into its components, color values were left as percentage transmission. Table I shows the results obtained on a number of soya

flours. For comparative purposes, bleached and unbleached wheat flours are included. The logarithm of percent transmission (log T) was then plotted against a percentage scale with the log T of full-fat soya taken as 100% and the relative pigment content of the various samples established on this basis.

TABLE I

COLOR VALUE OF EXTRACT OF SOYA AND WHEAT FLOURS

| | Transmission 440 mμ % | Log T | Relation to full-fat soya % |
|---|---|---|---|
| Full-fat soya No. 1 | 2.9 | 0.4624 | 100.0 |
| Solvent-extracted soya | 18.8 | 1.2742 | 47.0 |
| Bleached full-fat soya | 29.0 | 1.4624 | 34.0 |
| Unbleached soft wheat flour | 68.3 | 1.8344 | 10.5 |
| Bleached hard red winter wheat flour | 89.5 | 1.9518 | 2.5 |
| Solvent blank | 100.0 | 2.0000 | 0.0 |

With the samples studied, the full-fat soya flour has, roughly, 10 times the yellow pigment content of the unbleached soft wheat flour and 40 times that of the bleached hard red winter wheat flour used in this study. Since soya oil is yellow in color, it is not surprising to find that the solvent-extracted soya has only 47% as much yellow pigment as the full-fat type. The bleached full-fat flour shown in Table I was treated with 3 oz of Novadelox (a bleaching agent containing benzoyl peroxide) per 100 lb. Two thirds of the yellow pigment was removed. From the figures presented in the table, it can be determined that a flour blend containing 5% of bleached full-fat soya flour will have a relative yellow pigment value of 4.2 and will impart a creaminess to crumb color of bread equivalent to a medium bleached bread flour. The amount of Novadelox necessary to bleach full-fat flour has an improving effect on the baking qualities of wheat and soya flour mixtures.

The color of the extracted residue of soya flour also affects the crumb color of bread containing it, but methods for differentiating between small differences in color are not well established.

*Absorption.* There has been some confusion in the literature regarding the amount of water that a unit weight of soya flour will absorb when combined with wheat flour in the preparation of the dough. Bailey, Capen, and LeClerc (1935) disagreed with the claims for high absorption of soya flour when used with wheat flour in bread baking. Referring to soya flour prepared from the whole beans and the expeller process flour, they claim that the absorption was essentially the same as when no soya flour was used.

The absorption of a wheat flour and a combination of wheat and

soya was determined by the farinograph. The weight of dough was held constant at 480 g and absorption calculated from the resulting consistency. (Absorption of the mixture was calculated by using the experimentally determined factor 10 Brabender units = 0.3% absorption. Since the absorption of the wheat flour is known, the absorption of the soya flour can be readily calculated.) It required 199 g of water to bring 281 g of the flour that was used as a control to a consistency of 500 Brabender units.

As shown in Table II the addition of 3% and 5% full-fat soya flour,

TABLE II

ABSORPTION OF SOYA FLOUR–WHEAT FLOUR BLENDS

| Percent soya flour in blend | Consistency in Brabender units | Absorption of mix | Absorption of soya |
|---|---|---|---|
| | FULL FAT | | |
| % | | % | % |
| 0 | 500 | 70.8 | — |
| 3 | 490 | 70.5 | 60.0 |
| 5 | 500 | 70.8 | 70.8 |
| 10 | 530 | 71.7 | 80.8 |
| 20 | 590 | 73.5 | 86.8 |
| | SOLVENT EXTRACTED | | |
| % | | % | % |
| 0 | 505 | 70.8 | — |
| 3 | 530 | 71.5 | 95.8 |
| 5 | 550 | 72.1 | 96.8 |
| 10 | 590 | 73.2 | 94.8 |
| 20 | 740 | 77.5 | 104.0 |

based on the total weight of flour used, did not increase the absorption of the resulting dough. It was only when higher amounts (10% and 20%) of soya flour were used that any change was found in the consistency of the dough. The absorption of the soya flour was 86.8% in the case of the wheat-flour–20% full-fat soya flour blend.

The solvent-extracted soya flour has greater absorption than wheat flour. Thus we find that three and five parts of this type blended with wheat flour show an absorption of 95.8% and 96.8% for the soya flour. For all practical purposes, the solvent-extracted soya flour will absorb an amount of water equal to its weight when mixed with wheat flour to dough consistency. Doughs were also made of soya flour and water alone. They were premixed in the 3-qt. bowl of a Hobart mixer and 480 g transferred to the farinograph for a determination of consistency. The results were as follows:

| Absorption, % | 150 | 125 | 100 | 85 | 80 |
|---|---|---|---|---|---|
| Extracted, consistency, units | 275 | 400 | 650 | — | — |
| Full fat, consistency, units | — | — | 300 | 500 | 550 |

From this it can be estimated that the full-fat soya flour has an absorption at 500 units consistency of 85% and the solvent-extracted has an absorption of 110%. As the absorption of the full-fat flour is not much greater than wheat flour, it would not be expected that three parts soya flour blended with wheat flour would have any significant effect on the absorption of the resulting dough.

Different commercial brands of the same type soya flour do not vary significantly in their effect on absorption of wheat flour doughs when used to the extent of 5%. The values obtained on flour blends containing 5% full-fat soya flour from seven manufacturers varied from 70.0% to 71.4% absorption and of three blends containing extracted soya flours from 71.7% to 71.9%.

*Mixing Characteristic Studies.* A farinograph was used to study the effect of various types and different amounts of soya flour on mixing characteristics. To facilitate recording the results, the following points on this curve were used:

I *Initial Phase:* Time required for the top of the curve to reach the 500 consistency line.
M *Dough Development Stage:* Time required for the top of the curve to reach maximum height.
R *Range of Dough Stability:* Period of time the top of the curve stays above the 500 consistency line.
D *Breakdown:* Number of units drop below the 500 consistency line at the end of the test period, which in this case was 20 min.

The variation in mixing characteristics resulting from the use of 5% of several brands of full-fat soya flour is as follows:

| | I *min* | M *min* | R *min* | D *units* |
|---|---|---|---|---|
| Wheat flour | 4 | 6.5 | 11 | 65 |
| Wheat flour + 5% A | 2.5 | 6.5 | 11.5 | 55 |
| Wheat flour + 5% B | 3.5 | 6.5 | 11 | 55 |
| Wheat flour + 5% C | 3 | 6 | 12 | 55 |
| Wheat flour + 5% D | 3.5 | 6.5 | 10 | 65 |
| Wheat flour + 5% E | 3 | 7 | 11 | 55 |
| Wheat flour + 5% F | 3 | 6 | 12 | 50 |

When low-fat flours are used at the same level, the following results were obtained:

| | I *min* | M *min* | R *min* | D *units* |
|---|---|---|---|---|
| Low-fat soya A | 3 | 5.5 | 8 | 75 |
| Low-fat soya B | 3.5 | 6 | 8 | 75 |
| Low-fat soya C | 3 | 6 | 8 | 75 |

The difference between types as represented by full-fat and low-fat samples is much greater than the difference between brands. The low-fat flour shortens dough development time (M), reduces the range of dough stability (R), and increases the breakdown (D). Doughs con-

taining it will, therefore, have to be mixed a little less and there will be more danger of overmixing.

Since different brands vary in the amount of heat treatment they receive and this influences the color, nutritional value, and enzymatic activity, the effect of heat on mixing character is also important. Data obtained from farinograms of 5% blends of various samples ranging in heat treatment from low (*A*) to high (*D*) as reflected in color, *D* being the darkest, are as follows:

| | I *min* | M *min* | R *min* | D *units* |
|---|---|---|---|---|
| Wheat flour + soya flour A | 3 | 5.5 | 9 | 80 |
| Wheat flour + soya flour B | 3 | 6 | 10 | 60 |
| Wheat flour + soya flour C | 3.5 | 6.5 | 10 | 75 |
| Wheat flour + soya flour D | 3.5 | 6 | 9 | 80 |

There is some tendency for the more highly heated samples to give weaker curves, but it is certainly not a significant difference.

Since large percentages of soya flour have caused trouble in shop production and decreased bread quality, the effect of increasing amounts of soya flour on mixing character is of interest.

| | I *min* | M *min* | R *min* | D *units* |
|---|---|---|---|---|
| All wheat flour | 4 | 6.5 | 11 | 65 |
| 5% soya flour | 2.5 | 6.5 | 11 | 55 |
| 20% soya flour | 2.5 | 5.5 | 11 | 80 |

Small amounts have no adverse effect on the farinogram, but when 20 parts of soya flour are used, dough development time (*M*) is shortened and breakdown (*D*) is increased.

As shown below, the adverse effect of larger amounts of soya flour on mixing characteristics is not affected by the presence of yeast.

| | I *min* | M *min* | R *min* | D *units* |
|---|---|---|---|---|
| 20% full-fat soya | 2.5 | 5.5 | 11 | 80 |
| 20% full-fat soya + 3% yeast | 2.5 | 6 | 11 | 80 |

*Effect of Gassing Power.* Laufer, Tauber, and Davis (1944) have recently shown that soya beans are a good source of beta-amylase but contain only a trace of alpha-amylase. The effect of various types and amounts of soya flours was studied with the Blish-Sandstedt pressuremeter. Both undiastated flour, which was known to lack sufficient fermentable carbohydrates to sustain normal sponge fermentation, and a flour to which malted wheat flour had been added by the mill were used. The composition of the dough was: 10 g flour, 0.3 g compressed yeast, 7 ml distilled water, the water being increased by an amount calculated on the weight of the soya flour used in any one experiment. Fermentation was carried out at 30°C. The effect of varying amounts

of full-fat soya flour, solvent-extracted soya flour, and diastatically active malt syrup is compared below:

| Supplement added per 100 parts wheat flour | Total pressure in 6 hr. *mm* |
|---|---|
| None | 341 |
| 1 part malt syrup (20° Lintner) | 427 |
| 3 parts full-fat soya flour | 362 |
| 5 parts full-fat soya flour | 356 |
| 10 parts full-fat soya flour | 413 |
| 20 parts full-fat soya flour | 457 |
| 10 parts extracted soya flour | 427 |

It is evident that full-fat soya flour increases the gas production in doughs made from an undiastated flour, but the effect is slight when only 3% soya flour is included. Ten parts soya flour per 100 parts wheat flour has an effect similar to the use of 1% commercial malt (20° Lintner). This suggests that when high amounts of soya flour are used, malt syrup is not required in the formula for the purpose of developing fermentable carbohydrates. Soya flours are much richer in fermentable carbohydrates than wheat flour. Doughs made of various soya products were mixed with water and yeast and compared to wheat flour in the Blish pressuremeter with the following results:

| | Total pressure in 6 hr. *mm* |
|---|---|
| Undiastated wheat flour | 344 |
| Diastated wheat flour | 473 |
| Full-fat soya flour | 594 |
| Dehulled soya bean | 569 |
| Extracted soya flour | 770 |

The ground soya bean and the edible debittered soya flour prepared from it are a better source of fermentable carbohydrates than wheat flour, even when the latter contains added diastatic material. Extraction of the oil from the full-fat soya flour increases the activity of the residual flour because of the presence of more nonfat soya bean solids.

To investigate the relation between color of the soya flour and the rate of gas evolution from a fermenting dough, extracted soya flours of varying color were tested in increasing order of color, soya flour *A* being the lightest.

| | Total pressure in 6 hr. *mm* | Lintner value ° |
|---|---|---|
| 100 parts undiastated flour | 331 | — |
| 5 parts A—extracted soya flour (light) | 452 | 130–140 |
| 5 parts B—extracted soya flour | 427 | 10–15 |
| 5 parts C—extracted soya flour | 385 | nil |
| 5 parts D—extracted soya flour | 390 | nil |
| 5 parts E—extracted soya flour (dark) | 387 | nil |
| 5 parts full-fat soya flour | 397 | — |

The lightest-colored flour has the greatest effect on gas production, but there is not much difference in the three darkest flours, *C*, *D*, and *E*. These results correlate with the Lintner value of the samples.

The effect of varying amounts of soya flour on gas production of commercially diastated flour doughs is shown below:

| | Total pressure in 6 hr. mm |
|---|---|
| 0 parts soya flour | 493 |
| 3 parts full-fat soya flour | 494 |
| 5 parts full-fat soya flour | 498 |
| 10 parts full-fat soya flour | 526 |
| 20 parts full-fat soya flour | 564 |

Small amounts of soya flour (three parts per 100 parts wheat flour) have no significant effect on the rate of gas production. Malt syrup (20° Lintner) has much more effect. Large amounts (10–20%) of soya flour show greater gas production, reflecting the high saccharogenic activity compared to wheat flour. This suggests that in using either type of soya flour in commercial bread production with diastated flour, no adjustment need be made for any effect of soya flour on the rate of gas production. With 3% soya flour no change in the amount of yeast food in the dough is necessary.

*Lipoxidase Activity*. Sumner and Sumner (1940) presented evidence to show that the bleaching action of the raw soya bean is accomplished by the carotene oxidase enzyme and is an indirect result of the action of a fat peroxidizing enzyme on unsaturated fats. It is this effect of the raw soya flour which is utilized in the bleaching of wheat flour doughs. Lipoxidase is present in large quantities in the raw soya bean and in the flour prepared from it without heat treatment.

A method of determining lipoxidase activity by measuring the peroxide formed with linoleic acid and the amount of iron oxidized by it was suggested by Sumner (1943). Balls, Axelrod, and Kies (1943) outlined a method for assaying lipoxidase activity with carotene as an indicator, but this method appeared to have no advantages over Sumner's method for the purpose of the tests. The same was true of the method suggested by Reiser and Fraps (1943). The lipoxidase activity of various types of soya flour was determined by Sumner's method with the following results:

| | |
|---|---|
| Raw flour (unheated) | 35,000 units |
| Commercial extracted soya flour | 33.5 units |
| Commercial full-fat soya flour | 0 |

Baking tests were made employing the standard A.A.C.C. method to determine whether the lipoxidase activity of the soya flour had any effect on dough properties or bread quality aside from its bleaching

action. Five parts of full-fat soya flour were combined with wheat flour and varying amounts of raw soya bean flour added. The loaf volumes obtained in baking these samples were as follows:

| | Loaf volume *cc* |
|---|---|
| No raw flour | 490 |
| +.05% raw flour | 515 |
| +.10% raw flour | 500 |
| +.15% raw flour | 510 |
| +.20% raw flour | 510 |

Increasing amounts of the raw flour produced proportionately whiter crumb color, but no significant effect on loaf volume or other characteristics, including crumb grain, was apparent.

*Urease Activity.* Urease activity of different types and grades of soya flour varies, and it is important to evaluate the relation between urease activity of soya flour and its effect on baking properties of a bread dough.

The urease enzyme in soya flour would be of concern to the baker if urea was present in the dough. A patent in which soya flour is made part of a yeast food containing urea has been secured by Frey and Baker (1943); but urea is not present in a wheat flour dough and hence it is not to be expected that soya flour, because of its urease activity alone, would have an effect on the dough properties or bread quality. Bailey *et al* (1935) reported that soya flour of low urease content yielded bread of low volume while, on the other hand, every soya bean flour with high urease content produced a satisfactory loaf.

The relationship between urease activity and dough properties and bread quality was studied with several samples of soya flour which varied in urease activity from 1.6 to 120, as determined by the following method:

> Transfer 0.3 g of soya flour to a dry 800 ml Kjeldahl flask and add 250 ml tap water at 45°C; 50 ml 2% Urea solution, a few pieces of granulated zinc for an ebullator, and 1 drop mineral oil to prevent foaming. Stopper, mix well, and hold at 25°C for 3 hours with occasional stirring. Connect to Kjeldahl condenser and distill into 50 ml N/10 $H_2SO_4$ collecting about 100 ml distillate. Titrate excess acid with N/10 NaOH using sodium alizarin sulfonate as an indicator. Subtract blank; report as mg $NH_3$ per gram of sample per 3 hours.

The urease activity of several types of soya flour is shown below:

| | Urease activity *mg $NH_3$/g/3 hr.* |
|---|---|
| Soya flour, full-fat (not debittered) | 75.3 |
| Soya flour, full-fat (debittered) brand A | 20.4 |
| Soya flour, full-fat (debittered) brand B | 6.0 |
| Soya flour, solvent-extracted, light color | 120.3 |
| Soya flour, solvent-extracted, medium | 72.4 |
| Soya flour, solvent-extracted, dark | 1.6 |

The urease activity of nine commercial shipments of the same brand of full-fat soya flour made for a period of 8 weeks varied from 18.4 to 24.3 units.

Baking tests were carried out by the A.A.C.C. procedure on blends containing 5 parts of soya flour to 100 parts of wheat flour. Loaf characteristics, external and internal, and loaf volume were used as an index of the effect of soya flour. The urease content of the soya flours and the results of the baking tests are shown in Table III.

TABLE III

EFFECT OF UREASE ACTIVITY ON BREAD QUALITY

| | Urease activity | Basic loaf volume | Bromate[1] loaf volume | Loaf characteristics | |
|---|---|---|---|---|---|
| | | | | External | Crumb grain |
| | | cc | cc | | |
| 5% full-fat soya A | 6.0 | 500 | 505 | Smooth | Sl. spherical |
| 5% full-fat soya B | 20.4 | 490 | 520 | Smooth | Sl. spherical |
| 5% full-fat soya C | 59.0 | 510 | 505 | Smooth | Sl. spherical |
| Extracted soya, light | 120.3 | 505 | 535 | Smooth | Spherical |
| Extracted soya, medium | 72.4 | 470 | 495 | Smooth | Spherical |
| Extracted soya, dark | 1.6 | 505 | 485 | Smooth | Spherical |

[1] One mg potassium bromate per 100 g. of flour.

It would appear that while commercial grades of soya flour vary considerably in urease activity and this is apparently related to the amount of heat to which the soya flour has been exposed in processing, there is no correlation between urease activity and bread volume and quality. The effect of soya flour on baking properties must be accounted for in other ways. Urease activity can be disregarded in evaluating soya flour for all normal bread baking purposes.

*Effect on Dough Characteristics.* The effect of soya flour on dough characteristics and bread quality depends on the amount of soya flour blended with the wheat flour. This effect is similar to the action on dough of reducing substances such as glutathione and of proteolytic enzymes such as papain. In terms of the practical baker the bread would have the appearance of being "young." It is important to study this effect of soya flour on dough quality so that proper steps can be taken to compensate for it and research directed toward controlling it.

To study the gluten softening or proteolytic effect, mixing and resting tests were made using the farinograph in a manner described by Ford and Maiden (1938). Doughs were made of a blend of 20% full-fat soya flour with wheat flour, with and without compressed yeast. The consistency of these doughs was 500 F.U. when mixed to *M* (the maximum curve height). At the end of a rest period of 3 hours

at 30°C the dough was returned to the farinograph and its consistency at *M* again determined with the following results:

| | Dough consistency | | |
|---|---|---|---|
| | Flour-water | Flour-water-yeast | Commercial formula |
| | *F.U.* | *F.U.* | *F.U.* |
| 100 parts wheat flour | 410 | 330 | 320 |
| 100 parts wheat flour + 20% full-fat soya | 410 | 340 | 330 |
| Difference | 0 | +10 | +10 |

There is no significant difference in the decrease in consistency of the soya flour doughs in this time period compared to the wheat flour alone. Soya flour causes no softening of the gluten during fermentation such as is experienced when papain is present. From this it can be concluded that the action of soya flour is not due to a protease activating effect. If it were, the drop in farinograph consistency units would have been very great at the end of 3 hours' rest or fermentation period.

On the other hand, the softening effect of soya flour in a dough is more like the action of glutathione and appears to be an immediate effect on the flour proteins rather than as an activator of flour proteases. The shape of the farinograph curves made of wheat flour alone as a control, with a high percentage (20%) of soya flour, and with varying amounts of glutathione, the significant characteristics of which are shown below, leads to the belief that the action of soya flour is similar to glutathione.

| | I | M | R | D |
|---|---|---|---|---|
| | *min* | *min* | *min* | *units* |
| All wheat flour | 4 | 7 | 11.5 | 60 |
| All wheat flour + 20% full-fat soya | 2.5 | 5 | 11 | 80 |
| All wheat flour + 5 mg % glutathione | 3.5 | 4.5 | 10 | 110 |
| All wheat flour + 10 mg % glutathione | 3 | 4.5 | 7 | 135 |

To check this theory a series of doughs of similar composition was baked by the A.A.C.C. baking test procedure with the results shown in Table IV.

TABLE IV

COMPARATIVE EFFECTS OF GLUTATHIONE AND SOYA FLOUR ON BAKING CHARACTERISTICS

| | Loaf volume | Grain character |
|---|---|---|
| | *cc* | |
| Wheat flour | 620 | Elongated-attenuated |
| Wheat flour + 5 mg % glutathione | 570 | Spherical |
| Wheat flour + 10 mg % glutathione | 505 | Spherical, thick cell wall |
| Wheat flour + 15 mg % glutathione | 515 | Spherical, badly broken down |
| Wheat flour + 3% soya | 570 | Spherical |
| Wheat flour + 5% soya | 535 | Spherical |
| Wheat flour + 10% soya | 520 | Spherical, thick cell wall |

Doughs with glutathione produced the same type of loaf as soya flour additions. Loaf volume was affected adversely, oven spring was poor, the outside of the loaf was very smooth, the cell structure was spherical, and the texture was harsh.

Another series of baking tests showed that the effect of soya flour on dough properties was an immediate or direct one and hence, like glutathione, did not require time to manifest itself as in the case of papain. Using the A.A.C.C. test baking procedure, a comparison was made of the effect of varying fermentations on a hard wheat flour dough and on one in which 5% full-fat soya flour replaced an equivalent amount of wheat flour. The results are shown below:

| Fermentation time | 1 hour | 2 hours | 3 hours | 4 hours |
|---|---|---|---|---|
| Hard wheat flour, loaf volume | 610 cc | 670 cc | 645 cc | 595 cc |
| Hard wheat flour, crumb grain | Spherical | Well-developed | Well-developed | Well-developed |
| 5% full-fat soya, loaf volume | 550 | 590 | 595 | 505 |
| 5% full-fat soya, crumb grain | Spherical | Spherical | Spherical | V. spherical |
| Difference in loaf volume | −60 | −80 | −50 | −90 |

The difference in loaf volume between the bread made with and without soya flour is partly due to a dilution effect of the soya flour. Obviously, the same volume cannot be expected from a dough in which there is less gluten protein present. But aside from this, soya flour has an effect on volume that is maintained at a fairly uniform level below that of all wheat flour bread during the entire 4-hour fermentation period. The decrease in loaf volume at the end of 4 hours was due to lack of sufficient gassing power. The fact that the loaf volume of bread made with soya flour continued the same, or increased as long as gassing power was high, is further evidence that the action of soya flour is not proteolytic in nature. The weakening effect of soya flour on dough properties was noticed when the dough was punched at one hour, and the bread baked from the one-hour dough showed the characteristics associated with the use of soya flour.

The similarity in the effect of soya flour and glutathione on dough properties led to tests for −SH groups. Both full-fat and extracted soya flour gave positive nitroprusside tests for −SH groups. Quantitative tests for −SH groups were made using the method described by Stern (1944). The results on both types of soya flour are shown below, including nonactive brewer's yeast, a material which is known to be very drastic in its softening action on dough properties.

| | Total −SH [1] |
|---|---|
| Wheat flour | 0 |
| Full-fat soya | 14.4 |
| Full-fat bleached soya | 11.0 |
| Extracted soya, low heat | 10.2 |
| Extracted soya, high heat | 11.6 |
| Nonactive brewer's yeast | 40.4 |

[1] Expressed as ml M/600 $KIO_3$ per 10 g of flour.

As —SH groups are reducing in action, it is to be expected that oxidizing agents of the bromate and iodate type will improve the dough properties when soya flour is present and produce bread similar in character to wheat flour alone, except, of course, for volume which will continue to be affected by the dilution factor. The results of a series of baking tests using the A.A.C.C. procedure are as follows:

| | Loaf volume |
|---|---|
| | *cc* |
| Wheat flour | 585 |
| Wheat flour + 1 mg % K $BrO_3$ | 615 |
| Wheat flour + 5% full-fat soya | 550 |
| Wheat flour + 5% full-fat soya + 1 mg % K $BrO_3$ | 590 |
| Wheat flour + 5% full-fat soya + 0.5% $CaO_2$ | 595 |
| Wheat flour + 5% full-fat soya + 1 mg % $KIO_3$ | 595 |

The addition of oxidizing agents improved loaf volume and internal and external characteristics of the bread made with soya flour. It was comparable in quality to bread made without soya. Benzoyl peroxide used to bleach soya flour had a somewhat similar effect.

To overcome the effect of soya flour on dough quality, these results suggest that dough improvers of the bromate or iodate type should be used in amounts necessary to obtain optimum bread. Actual baking experience indicates these amounts are higher than commonly used. It would be more desirable from the standpoint of the baker if the soya flour were treated by the manufacturer with the proper oxidizing material so it could be used by him with no disturbance of regular fermentation or change in the amount of bread improver.

*Effect on Rate of Staling.* To determine the effect of soya flour on the rate of staling of bread, two full-size commercial doughs were prepared by the sponge dough process under laboratory supervision. The formulas were identical except that one contained no soya flour and was the regular production formula; the other contained three parts of full-fat soya flour to each 100 parts of flour, plus two and a half parts of water to provide for the absorption of the soya flour. The doughs were placed in the middle of a day's run and were handled in the usual manner in normal sequence of production. The bread was baked in an oil-fired traveling oven without any breaks between doughs so that oven temperatures and baking times were constant.

Several rows of bread extending across the oven were selected from the regular and special doughs. This bread was cooled, sliced in the usual manner, and wrapped in opaque wax paper. Cooling time and conditions were kept constant. Eighteen loaves were selected at random from each of these two groups and stored at 70°F and 40% relative humidity. Six loaves were taken each day for compressibility tests and six slices, selected from the center portion of each loaf, were

tested using the Wallace-Tiernan compressimeter described by Platt and Powers (1940). A 1½ inch square was cut from the center of each slice and the stress in grams required to compress it 2.5 mm was noted. The average of the test for six loaves, or 36 slices, was taken as a measure of the compressibility. This test was made at the end of 24, 48, and 72 hours. The results were as follows:

| Age | Grams stress per 2.5 mm strain | |
|---|---|---|
| | Control | 3% soya flour |
| 24 hours | 8.9 | 8.5 |
| 48 hours | 13.8 | 13.6 |
| 72 hours | 20.0 | 17.0 |

The bread containing soya flour is more compressible than the regular loaf and continues so throughout the period of the test of 72 hours. Furthermore, there is a much slower rate of staling between the third and first day with the bread containing soya flour.

## Summary

Soya flour was found that contained rope spores well below a maximum of 50 per gram. No trouble was experienced with rope in bread made from such soya flour under conditions normally used to control rope development.

A typical full-fat soya flour had 10 times the yellow pigment content of a sample of unbleached soft wheat flour but two thirds of this color was readily removed by treatment with high amounts of benzoyl peroxide (3 oz Novadelox per 100 lb of flour). These amounts had an improving effect on the baking qualities of wheat and soya flour mixtures.

The absorption of full-fat and solvent-extracted flour, as determined by the Brabender farinograph, was found to be 85% and 110% respectively. Different commercial brands of soya flour did not vary significantly in their effect on the absorption of wheat flour doughs.

Farinograph studies of dough properties during mixing showed that low-fat flour shortened dough development time, reduced range of dough stability, and increased the breakdown upon prolonged mixing. Less mechanical modification is suggested with such flour. Full-fat soya flour had less effect than low-fat on the breakdown of the dough. Different brands do not vary significantly in their effect on the character of the farinogram. Treatment of the beans with different degrees of heat to produce a debittered flour does not significantly affect the farinograph curve.

Full-fat soya flour is a much better source of fermentable carbohydrates than wheat flour; but when only 3% of soya flour is blended with wheat flour, the effect on gas production may be neglected.

Diastatic supplements such as malt syrup or malted wheat flour can be maintained at the same levels as ordinarily used.

The lipoxidase activity of soya flour is high when produced from the unheated raw soya bean. In the amounts used to accomplish the desired bleaching action in a wheat flour dough, lipoxidase content of raw soya bean flour has no effect on the volume, external characteristics, or crumb and grain of the bread.

Commercial grades of soya flour vary considerably in their urease activity. This is apparently related to the amount of heat to which the soya flour has been exposed in the debittering treatment. No correlation was found between urease activity and quality of bread.

The effect of soya flour on fermenting dough without any compensating factors depends on the amount of soya flour used and is similar to the action of reducing substances such as glutathione and proteolytic enzymes such as papain. Mixing and resting tests using the farinograph indicate that the effect of soya flour on gluten protein is not a protease-activating or a proteolytic effect but is similar in nature to that of glutathione. Oxidizing agents of the bromate and iodate type improve the dough properties when soya flour is present; and when used in sufficient quantity, they produce bread similar in character to wheat flour except for volume which is affected by the dilution factor.

Bread made with soya flour is more compressible and the rate of staling is slower than for bread produced under similar conditions from wheat flour alone.

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## AMINO ACID COMPOSITION OF GRAIN ALCOHOL FERMENTATION BY-PRODUCTS [1]

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Interest in the amino acid composition of protein foods and feeds has been heightened greatly by the shortage of animal proteins. Many plant proteins are known to be deficient in one or more essential amino acids. Grain alcohol fermentation by-products play a prominent role (Bauernfeind and Boruff, 1944) in animal feeding, particularly since nonfat milk solids, meat scraps, and similar materials became scarce owing to war shortages. The vitamin content (Bauernfeind, Garey, Baumgarten, Stone, and Boruff, 1944; Bauernfeind, Smith, Garey, Baumgarten, Gustoff, and Stone, 1944) of these products is well established; however, the amino acid content (Joseph, Kolachov, and Willkie, 1942) has been investigated only briefly.

Determination of amino acids by chemical methods has been carried out for a long time, but the close structural similarity of several of the natural amino acids renders these methods unreliable. Microorganisms are known to be fastidious in their nutritive requirements, and it was established that growth and multiplication depended on the availability of specific nutrilites. The vitamin requirements of a number of microorganisms are well established and use is made of this property in microbiological assays of vitamins. Recently the amino acid requirements of two lactobacilli, *Lactobacillus arabinosus* 17–5 (Hegsted, 1944; Kuiken, Norman, Lyman, Hale, and Blotter, 1943; Shankman, 1943; Shankman, Dunn, and Rubin, 1943) and *Lactobacillus*

[1] Portions of this material were presented before the Division of Biological Chemistry, 108th Meeting of the American Chemical Society, New York, September 11–15, 1944.

*casei* (Hutchings and Peterson, 1943; McMahan and Snell, 1944; Shankman *et al*, 1943a), were described and applications to amino acid analyses were indicated.

In the present paper the determination of 10 amino acids in alcohol fermentation by-products from grain is reported. The microbiological assays are described in some detail because difficulties are occasionally experienced.

## Microbiological Technique

Stab cultures of *L. arabinosus* and *L. casei* were carried in 1% glucose, 1% yeast extract, and 1.5% agar medium. The inoculum was prepared according to Snell and Wright (1941) and incubated for 18 to 24 hours at 37°C before use. The medium (Table I) was reported recently (Baumgarten, Garey, Olsen, Stone, and Boruff, 1944); it was employed with both *L. arabinosus* and *L. casei*. (The amino acids used were obtained from B. L. Lemke Company.) Use was made of *Streptococcus lactis* R (Snell and Guirard, 1943) for the determination of several amino acids not determinable with either *L. arabinosus* or *L. casei* in which case 0.2 mg of xanthine per assay tube was added to the medium.

Hydrolyzed casein supplemented with cystine and tryptophane contains all the amino acids essential for the nutrition of these organisms and is therefore utilized in testing their growth response. The casein hydrolysate was prepared either by refluxing with hydrochloric acid (1 : 1) or by autoclaving with sulfuric acid (25% by volume) for 12 hours (Williams, 1941).

It was established that maximal growth response of *L. arabinosus* and *L. casei*, as measured by lactic acid production, was obtained if the nitrogen requirements were supplied either by hydrolyzed casein supplemented with cystine and tryptophane or by the 20 amino acids in the medium (Table I). Maximal growth of homofermentative lactic acid bacteria as used by the authors implies near quantitative conversion of the glucose employed into lactic acid. Experiments have been carried out to substantiate this claim under the conditions employed.

The growth of *S. lactis* R is determined turbidimetrically and therefore the measurement employed is the comparison of growth support by supplemented hydrolyzed casein with individual amino acids.

Cystine, glutamic acid, isoleucine, leucine, methionine, threonine, tryptophane, and valine were found to be essential for the growth of *L. arabinosus*, whereas arginine, cystine, glutamic acid, isoleucine, leucine, phenylalanine, serine, tryptophane, tyrosine, and valine were found to be essential amino acids for *L. casei*. In addition to these essential amino acids the other nutrilites in Table I must be supplied

for satisfactory growth. The single omission of any one of the essential amino acids from the medium containing 20 amino acids would prevent practically all growth. Irregular results prevented the establishing of a curve for cystine with *L. casei*. Commercially available amino acids are often contaminated with their isomers or closely related compounds (Schmidt, 1938). *dl*-Leucine was reported by Hegsted and Wardwell (1944) to contain isoleucine. The present authors found that 2 mg *l*(−)-leucine, the amount present in the medium per assay tube, contained sufficient methionine and isoleucine to satisfy the needs of the organisms for these nutrilites. However, by substituting *dl*-leucine (Lemke & Company), satisfactory standard curves for methionine and

TABLE I

COMPOSITION OF MEDIUM FOR MICROBIOLOGICAL ASSAYS OF AMINO ACIDS
(Per tube of 10 ml)

| | | | |
|---|---|---|---|
| Glucose | 100 mg | *dl*-alanine | 2 mg |
| Sodium acetate anhyd. | 60 mg | *l*(+)-arginine | 2 mg |
| Adenine | 0.5 mg | Asparagine | 2 mg |
| Guanine | 0.5 mg | *l*(−)-cystine | 2 mg |
| Uracil | 0.5 mg | *l*(+)-glutamic acid | 2 mg |
| Salt A [2] | 0.5 ml | glycine | 2 mg |
| Salt B [2] | 0.5 ml | *l*(−)-histidine | 2 mg |
| Thiamine hydrochloride | 2 γ | *l*(−)-hydroxyproline | 2 mg |
| Nicotinic acid | 4 γ | *dl*-isoleucine | 2 mg |
| Riboflavin | 2 γ | *l*(−)-leucine | 2 mg |
| Pyridoxine hydrochloride | 2 γ | *dl*-lysine | 2 mg |
| *p*-Aminobenzoic acid | 2 γ | *dl*-methionine | 2 mg |
| Calcium-pantothenate | 2 γ | *dl*-norleucine | 2 mg |
| Biotin | 0.05 γ | *dl*-phenylalanine | 2 mg |
| Folic acid | 4500 γ [1] | *l*(−)-proline | 2 mg |
| | | *dl*-serine | 2 mg |
| | | *dl*-threonine | 2 mg |
| | | *l*(−)-tryptophane | 2 mg |
| | | *l*(−)-tyrosine | 2 mg |
| | | *dl*-valine | 2 mg |

[1] Potency *l* material for which we wish to thank Dr. R. J. Williams of the University of Texas.
[2] Williams (1941).

isoleucine were established. *dl*-Tryptophane was employed in assaying tyrosine since *l*(−)-tryptophane contained considerable amounts of tyrosine. Standard curves for arginine, lysine, phenylalanine, and tyrosine (Hegsted, 1944; Kuiken *et al*, 1943) were not obtained when *L. arabinosus* was the test organism; under identical conditions such curves were established for arginine, phenylalanine, and tyrosine with *L. casei* and for lysine with *S. lactis* R.

The samples for analysis were prepared by refluxing for 24 hours with constant boiling hydrochloric acid. It was found advantageous to preheat the samples on the water bath for 12 hours in order to diminish the formation of black humin deposit in the reflux condenser.

After completion of the hydrolysis the samples were filtered, concentrated in vacuo, and stored in the refrigerator. The medium (Table I), from which the assayed amino acid was omitted, was adjusted to pH 6.8–7.0 and diluted to contain the nutrilites for one assay tube in 5 ml of solution. Suitable aliquots of the assayed amino acid or of the neutral hydrolysate are added to the culture tubes (25 × 200 mm) each containing 5 ml of the above medium. Standard curves are established each time an assay is carried out. The amount of the different amino acids that support maximal growth varies with each amino acid.

After autoclaving and inoculation, the samples were incubated for 72 hours at 30°C with *L. arabinosus* and *S. lactis* R and at 37°C with *L. casei*. The growth response was measured by titration of the lactic acid formed in the assays with *L. arabinosus* and *L. casei*. *S. lactis* R did not produce large enough amounts of lactic acid under our experimental conditions. Its growth response was measured turbidimetrically by determining the absorption of the culture medium at 620 mμ with a Coleman Universal Spectrophotometer.

Arginine, glutamic acid, histidine, isoleucine, leucine, lysine, phenylalanine, threonine, tyrosine, and valine were determined. Arginine, phenylalanine, and tyrosine were determined with *L. casei;* threonine was determined with *L. arabinosus* and *S. lactis* R; histidine and lysine were determined with *S. lactis* R; glutamic acid, isoleucine, leucine, and valine were determined with both *L. casei* and *L. arabinosus*. Microbiological values obtained with different assay organisms agree well. A study comparing amino acid determinations with several microorganisms was undertaken and the results will be presented at a later time. In addition, methods of determination for cystine, methionine, serine, and tryptophane are available. Preliminary results were obtained on these amino acids, but certain difficulties are encountered in preparing satisfactory hydrolysates for analyses of the four amino acids.

The naturally occurring *l* forms of the amino acids were used in establishing the standard curves for arginine, glutamic acid, histidine, leucine, lysine, and tyrosine; the *dl* forms of isoleucine, phenylalanine, threonine, and valine were employed for standard curves with these amino acids. In all instances it was assumed that the hydrolysates contained only the naturally occurring or *l* forms of the amino acids. All values are expressed in terms of the *l* amino acid. If the *dl* acid was used as the standard, its activity was taken at 50% of that of the *l* form (Stokes and Gunness, 1944).

Humin formation is responsible for losses of amino acid during hydrolysis. However, it has been reported that losses of isoleucine, leucine, and valine (Kuiken *et al*, 1943) are negligible.

Recovery experiments were carried out. The amino acid was added

to the sample. Two samples, one containing the added amino acid, were hydrolyzed under identical conditions. Recovery was calculated from the values of the particular amino acid obtained on analysis of the two preparations and expressed on a percentage basis. Recovery was satisfactory and amounted to 95 to 105% of the added amino acid with the exception of tyrosine which was recovered to about 80%. Considerable destruction of tyrosine must be assumed during hydrolysis inasmuch as it has been reported (Lugg, 1938) sensitive to acid treatment. Recovery experiments with free amino acids in a similar fashion to determine the stability of amino acids during hydrolysis have been employed previously and judged reliable (Schweigert, McIntire, Elvehjem, and Strong, 1944).

In carrying out biological assays it must be kept in mind that precursors or metabolites of the assayed substances may show activity. For instance, Greene and Black (1943) have found that indole and anthranilic acid could replace tryptophane as a nutrilite for *L. arabinosus*. Preliminary experiments on tryptophane determinations of the materials examined indicated that only inappreciable amounts of such interfering substances which possess tryptophane activity were present. Glutamic acid was determined and found to be present in comparable amounts to those established by microbiological assay.

## By-Product Analysis

The materials examined were distillers' dried solubles, distillers' dried grains with solubles (dark grains), distillers' dried grains or screenings (light grains), and centrifuge cake. Distillers' dried grains or screenings comprise the coarse particles of the whole stillage which are screened through a 0.75 mm screen; distillers' dried solubles are the dried clarified liquid after centrifuging (Boruff and Miller, 1938; Cooley, 1938); distillers' dried grains with solubles contain the whole stillage, whereas the centrifuge cake is the solid portion removed during centrifuging.

Grain alcohol is produced from a variety of sources. Consequently the amino acid content of distillery by-products from different mash bills was determined. In addition the raw materials of the mash bills were analyzed. Table II presents the amino acid composition of distillers' dried solubles. Arginine and glutamic acid differ between the various mash bills, but only slight variation is observed with the other amino acids. Distillers' dried grains with solubles show a similar relationship as noted from Table III. These results should be expected as glutamic acid and arginine occur in greater percentage in wheat than in corn, and glutamic acid is observed present in larger amounts in by-products derived mainly from wheat. In Table IV the amino acid

TABLE II

AMINO ACID CONTENT OF DISTILLERS' DRIED SOLUBLES PRODUCED FROM VARIOUS MASH BILLS

| Amino acid | Dry matter basis | | |
|---|---|---|---|
| | *A* | *B* | *C* |
| | % | % | % |
| Arginine | 0.6 | 0.9 | 1.1 |
| Glutamic acid | 5.6 | 9.8 | 11.2 |
| Histidine | 0.7 | 0.9 | 0.8 |
| Isoleucine | 1.5 | 1.8 | 1.8 |
| Leucine | 1.4 | 1.5 | 1.5 |
| Lysine | 0.7 | 0.8 | 0.7 |
| Phenylalanine | 1.7 | 1.9 | 2.1 |
| Threonine | 0.9 | 1.1 | 1.0 |
| Tyrosine | 0.6 | 0.9 | 0.8 |
| Valine | 1.4 | 1.8 | 1.5 |
| | *A* | *B* | *C* |
| Composition of mash bill | 87% Corn<br>3% Rye<br>10% B. Malt | 23% Corn<br>54% Wheat<br>10% G.W.F.[1]<br>3% Rye<br>10% B. Malt | 70% Wheat<br>17% G.W.F.[1]<br>3% Rye<br>10% B. Malt |

[1] Granular wheat flour.

composition of corn, wheat, granular wheat flour, rye, and barley malt is presented.

There is no indication that the protein of the raw materials undergoes extensive changes in its amino acid composition. The percentage

TABLE III

AMINO ACID CONTENT OF DISTILLERS' DRIED GRAINS WITH SOLUBLES PRODUCED FROM VARIOUS MASH BILLS

| Amino acid | Dry matter basis | | |
|---|---|---|---|
| | *A* | *D* | *C* |
| | % | % | % |
| Arginine | 0.9 | 1.2 | 1.2 |
| Glutamic acid | 7.1 | 8.8 | 10.1 |
| Histidine | 0.8 | 0.9 | 0.9 |
| Isoleucine | 2.0 | 2.5 | 1.9 |
| Leucine | 2.4 | 2.1 | 1.4 |
| Lysine | 0.7 | 0.9 | 0.8 |
| Phenylalanine | 2.1 | 2.1 | 2.2 |
| Threonine | 1.1 | 1.2 | 1.0 |
| Tyrosine | 0.7 | 0.7 | 0.7 |
| Valine | 1.8 | 2.2 | 1.9 |
| | *A* | *D* | *C* |
| Composition of mash bill | 87% Corn<br>3% Rye<br>10% B. Malt | 28% Corn<br>48% Wheat<br>11% G.W.F.[1]<br>3% Rye<br>10% B. Malt | 70% Wheat<br>17% G.W.F.[1]<br>3% Rye<br>10% B. Malt |

[1] Granular wheat flour.

of amino acid present in corn, wheat, granular wheat flour, rye, and barley malt was compared to that in distillers' dried grains with solubles. As this material comprised the whole stillage it corresponded most closely to the raw materials, taking into account that the starch is removed during fermentation. The found and calculated values are compared in Table V. It must be kept in mind that a considerable concentration of the protein fraction has taken place and a factor should be applied to compare the magnitude of the amino acids in the mash bill with that in the final products. The factor depends on the composition of the mash bill as the protein content and fermentable starch of the raw materials vary. It is therefore advisable to compare

TABLE IV

AMINO ACID CONTENT OF RAW MATERIALS COMPRISING THE MASH BILLS

| Amino acid | Raw material (dry matter basis) | | | | |
|---|---|---|---|---|---|
| | Corn [1] | Wheat [2] | Granular wheat flour [3] | Rye [4] | Barley malt [5] |
| | % | % | % | % | % |
| Arginine | 0.5 | 0.6 | 0.5 | 0.6 | 0.4 |
| Glutamic acid | 2.5 | 6.3 | 5.3 | 3.5 | 2.8 |
| Histidine | 0.3 | 0.5 | 0.4 | 0.4 | 0.3 |
| Isoleucine | 0.6 | 1.0 | 0.9 | 0.7 | 0.7 |
| Leucine | 0.8 | 0.8 | 0.8 | 0.6 | 0.6 |
| Lysine | 0.3 | 0.5 | 0.3 | 0.3 | 0.4 |
| Phenylalanine | 0.6 | 0.7 | 0.8 | 0.7 | 0.7 |
| Threonine | 0.3 | 0.5 | 0.3 | 0.3 | 0.4 |
| Tyrosine | 0.1 | 0.2 | 0.2 | 0.2 | 0.1 |
| Valine | 0.7 | 0.7 | 0.6 | 0.8 | 0.8 |

[1] Illinois hybrid corn.
[2] Hard red winter wheat.
[3] Milled from hard red winter wheat.
[4] North west plump rye.
[5] Distillers' grade malted barley.

the amount of nitrogen accounted for by the individual amino acids instead of the percentage present. This eliminates the necessity of depending on a conversion factor which also varies with recovery data. By taking into account that values determined microbiologically are accurate within 5 to 10%, only the tyrosine content shows considerable difference between calculated and found values. This might be attributed to synthesis or to increased destruction of tyrosine during the hydrolysis of the mash bill grain samples, which is plausible as only these materials contain considerable amounts of carbohydrate material and therefore greater humin formation is to be expected. The data bear out the fact that the amino acid composition of distillers' by-products can be predicted with a fair degree of accuracy from the composition of the mash bill.

The distribution of the amino acids in the protein of the different fractions of alcohol fermentation by-products is nearly the same, if the per cent of nitrogen accounted for is taken into consideration. The percentage present of the individual amino acids varies with the products as the protein content varies. Centrifuge cake is the richest source of protein, whereas distillers' dried solubles, distillers' dried grains with solubles, and distillers' dried grains contain approximately identical amounts of protein. In Table VI the values of the amino acids observed in the different fractions are compiled.

TABLE V

COMPARISON OF AMINO ACID CONTENT OF DISTILLERS' DRIED GRAINS WITH SOLUBLES AS FOUND AND AS CALCULATED FROM THE COMPOSITION OF THE INDIVIDUAL CONSTITUENTS OF THE MASH BILL

| Amino acid | Nitrogen accounted for | | | | | |
|---|---|---|---|---|---|---|
| | A | | D | | C | |
| | Found | Calculated | Found | Calculated | Found | Calculated |
| | % | % | % | % | % | % |
| Arginine | 5.9 | 7.5 | 5.9 | 6.6 | 5.6 | 6.2 |
| Glutamic acid | 13.1 | 12.2 | 13.1 | 17.4 | 14.5 | 19.1 |
| Histidine | 4.2 | 4.7 | 3.8 | 4.2 | 3.7 | 4.0 |
| Isoleucine | 4.2 | 3.3 | 4.2 | 3.4 | 3.1 | 3.5 |
| Leucine | 5.0 | 4.2 | 3.6 | 3.2 | 2.2 | 2.9 |
| Lysine | 2.6 | 3.2 | 2.6 | 3.0 | 2.2 | 3.0 |
| Phenylalanine | 2.7 | 2.8 | 3.4 | 2.3 | 2.8 | 2.1 |
| Threonine | 2.5 | 1.6 | 1.9 | 2.2 | 1.7 | 1.8 |
| Tyrosine | 1.1 | 0.5 | 0.9 | 0.5 | 0.8 | 0.5 |
| Valine | 4.3 | 4.3 | 4.2 | 3.4 | 3.4 | 3.1 |

| | A | D | C |
|---|---|---|---|
| Composition of mash bill | 87% Corn<br>3% Rye<br>10% B. Malt | 28% Corn<br>48% Wheat<br>11% G.W.F.[1]<br>3% Rye<br>10% B. Malt | 70% Wheat<br>17% G.W.F.[1]<br>3% Rye<br>10% B. Malt |

[1] Granular wheat flour.

Only a few values are available in the literature on the amino acid composition of distillers' by-products. Joseph *et al* (1942) determined the arginine, histidine, and lysine content of distillers' dried solubles from a corn-wheat mash bill. Our data on a similar mash bill show that 5% of the protein is accounted for by arginine, 4% by histidine, and 3% by lysine, whereas the respective data of Joseph *et al* are 6.05%, 2.83%, and 6.24%. Block and Bolling (1944) reported data on the amino acid composition of whole corn and wheat grains. The discrepancies with regard to leucine content are considerable. We found

TABLE VI

COMPARISON OF THE AMINO ACID DISTRIBUTION IN VARIOUS PRODUCTS FROM THE SAME MASH BILL

| Amino acid | By-product (dry matter basis) | | | |
|---|---|---|---|---|
| | Dried grains with solubles | Dried solubles | Dried screenings | Dried centrifuge cake |
| | % | % | % | % |
| Arginine | 1.2 | 1.1 | 1.2 | 1.9 |
| Glutamic acid | 10.1 | 11.2 | 8.8 | 15.8 |
| Histidine | 0.9 | 0.8 | 0.9 | 1.4 |
| Isoleucine | 1.9 | 1.8 | 2.2 | 3.4 |
| Leucine | 1.4 | 1.5 | 1.8 | 2.9 |
| Lysine | 0.8 | 0.7 | 0.8 | 1.2 |
| Phenylalanine | 2.2 | 2.1 | 1.8 | 2.9 |
| Threonine | 1.0 | 1.0 | 1.0 | 1.7 |
| Tyrosine | 0.7 | 0.8 | 0.5 | 1.2 |
| Valine | 1.9 | 1.5 | 1.9 | 3.0 |
| Total protein ($N \times 6.25$) | 41.8 | 40.9 | 43.7 | 59.3 |
| Composition of mash bill | 70% Wheat<br>17% Granular wheat flour<br>3% Rye<br>10% Barley malt | | | |

for corn 4.5% of the protein to be composed of leucine to 22% by Block and Bolling, whereas for wheat our figure is 2.9% as compared to 13% by Block and Bolling. There is also considerable difference in the tyrosine content; however, we believe that our values might be too low due to the method of hydrolysis.

## Summary

Microbiological assay methods for arginine, glutamic acid, histidine, isoleucine, leucine, lysine, phenylalanine, threonine, tyrosine, and valine are reported.

The amino acid content of grain alcohol fermentation by-products from different mash bills and for the original grains was determined. It was established from these data that the amino acid composition of grain alcohol by-products can be predicted from the raw materials.

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# INFLUENCE OF DOUGH CONSTITUENTS ON FERMENTATION

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The rate of alcoholic fermentation in bread dough has heretofore not been equalled in solutions of known composition. Simpson (1936) compared the rate of fermentation in Hansen's yeast nutrient solution (Jörgensen, 1925), and Slator's solution (1906, 1908) with a flour-water mixture. He found that in Hansen's solution yeast fermented at a higher rate than in Slator's solution, but the rate in the fermenting dough was higher yet—to the extent of about 75%. Hansen's solution contained peptone and thus cannot be accurately described as being of known composition. The attempt to reproduce dough fermentation rates with solutions of known composition (*i.e.* so-called synthetic solutions) is made because it appears to be a necessary first step toward a more complete knowledge of the factors in a dough that influence the rate of gas production.

Since the dough supports a much higher fermentation rate than a simple salt solution, it would appear that flour contains a substance or substances which exert a powerful stimulating influence on the rate of alcoholic fermentation. The existence of such a substance in nature has long been suspected. Euler and Swartz (1924) gave the designation "Factor Z" to this substance which they found to be relatively widespread in extracts of plant and animal tissues. Schultz, Atkin, and Frey (1937) discovered that vitamin $B_1$ (thiamine) has the properties of "Factor Z" and, in fact, is indistinguishable from it. Thiamine occurs in flour in an amount adequate to stimulate fermentation (Schultz *et al*, 1939b). Investigation has shown that a solution containing potassium, magnesium, phosphate and sulfate ions, thiamine, pyridoxine, nicotinic acid, a source of yeast-available nitrogen, sugar, and a sodium citrate buffer is able to support a rate of fermentation equal to that of a flour suspension fortified with an excess of sugar and available nitrogen.

It is the object of the present report to describe the specific effect of each of the above factors on fermentation rates. A detailed study of the influence of individual factors will form the subject of later communications.

## Materials and Methods

The principal piece of apparatus employed is the fermentometer described by Schultz *et al* (1942). The fermenting solutions are

contained in bottles of 8 oz (250 ml) capacity. The bottles are partially immersed in a water bath at 30°C. A shaking mechanism swings the bottles through an arc at about 100 complete excursions per minute. In other work of this sort it has been determined that the rate and amplitude of shaking is satisfactory so long as the yeast does not settle out of suspension. Too violent shaking should be avoided. The gas evolved is collected in 500 ml gasometers over a 10% calcium chloride solution. The smallest graduation on the gasometer is equivalent to 5 ml and consequently a precision of greater than 1 to 2 ml cannot be expected.

Individual aqueous solutions of the various compounds are sterilized in flowing steam for 30 minutes and thereafter stored at room temperature. The concentrations chosen are such that all of the ingredients of the "complete" mixture required for 20 ml of the final medium will be contained in 8 ml of solution. If no other additions are made, this leaves room for 2 ml of water and 10 ml of yeast suspension. The yeast suspension is prepared before each run by suspending 6 g of fresh bakers' yeast in 100 ml of water.

All ingredients, except yeast and flour, are placed in the reaction bottles. Two minutes before "zero time" the pipetting of the yeast suspension is begun. If flour is to be added, it is stirred into the mixture after the yeast has been added. The bottles are capped and placed in the thermostat as quickly as possible (usually at "zero time"). After allowing 2 minutes for temperature equilibrium, readings are made of the levels in the gasometers. This is the initial or "zero reading." The volumes are then measured every 15 minutes.

## Results

In Figure 1 the rate of fermentation of a "complete" synthetic solution is compared with that of flour paste which has been fortified with salt and an excess of nitrogen in the form of asparagine.[1] After an initial lag in gas evolution a more or less constant rate of acceleration appears in the "complete" mixture which continues for the 3-hour period. Flour fortified with salt and asparagine shows a slightly greater lag, but then gas evolution follows the same course as in the "complete" solution. The tests with sugar and water only and with sugar and water plus salt and asparagine show no acceleration but rather an almost-constant rate after the initial lag. The initial lag in all cases is considered to be due, in part, to a short induction period and also to initial gas retention by the fermentation medium. These

[1] Since there is no evidence that gas evolution is discontinuous, an attempt is made to draw the best curves through the points given by the experimental data. This procedure has been generally adopted in the experiments reported below, except where crowding necessitates omission of the line.

experiments were not continued past 180 minutes because it was desired to avoid complications due to sugar exhaustion which might occur when about 400 ml of gas had been evolved.

*Influence of Phosphate, Sulfate, Magnesium, and Potassium.* The influence of each of these factors is demonstrated by the omission of

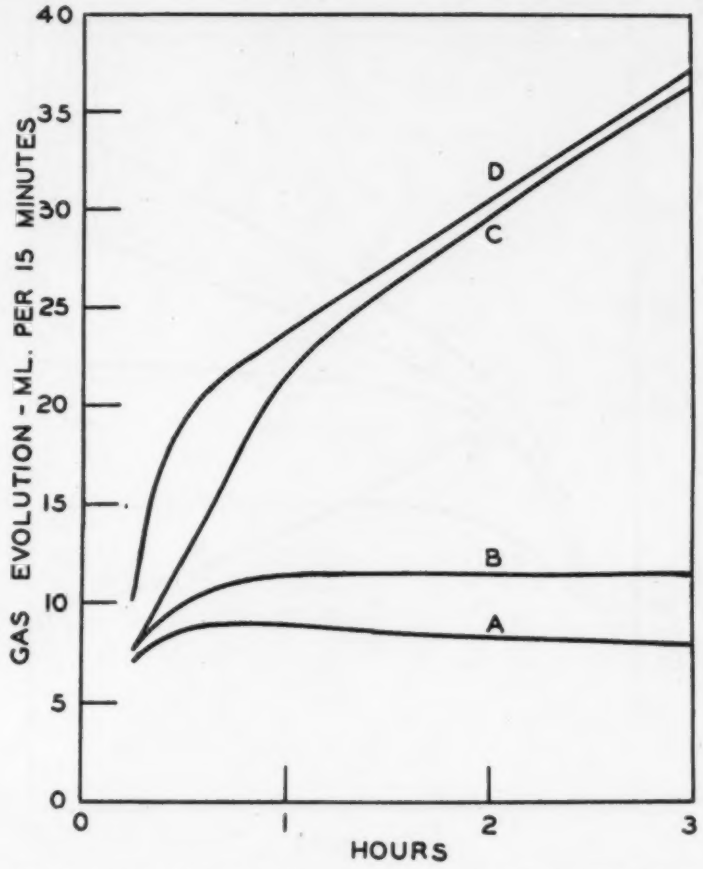


Fig. 1. Influence of various additions upon the fermentation of a sucrose solution.

A. Control (sucrose 2 g, yeast 0.6 g, water to 20 ml, Temp. 30°C).
B. Salt and asparagine (same as A, plus NaCl 0.2 g, asparagine 0.2 g).
C. Flour, salt, and asparagine (same as A, plus NaCl 0.2 g, asparagine 0.2 g, white flour 20 g).
D. "Synthetic" mixture (same as A, plus $NaH_2PO_4.H_2O$ 0.06 g, $MgSO_4.7H_2O$ 0.04 g, K Cl 0.01 6 g asparagine 0.2 g, thiamine 0.00008 g, pyridoxine 0.00008 g, nicotinic acid 0.0008 g, and sodium citrate buffer (M/3) pH 5.5 2.4 ml).

each in turn from the "complete" synthetic medium. The results of these experiments are shown in Figure 2. With no phosphate no acceleration is observed and the rate does not rise above 15 ml per 15 minutes. With no sulfate the rate attained is higher (about 20 ml), but again acceleration is lacking. When magnesium is omitted an initial rise in rate is followed by a relatively sharp drop. When there

is no potassium in the mixture a fairly high fermentation rate is developed and also an acceleration, but the rate is always significantly lower than in the "complete" mixture. The separate omission of magnesium and sulfate was accomplished by the substitution of sodium sulfate or magnesium lactate for magnesium sulfate. A

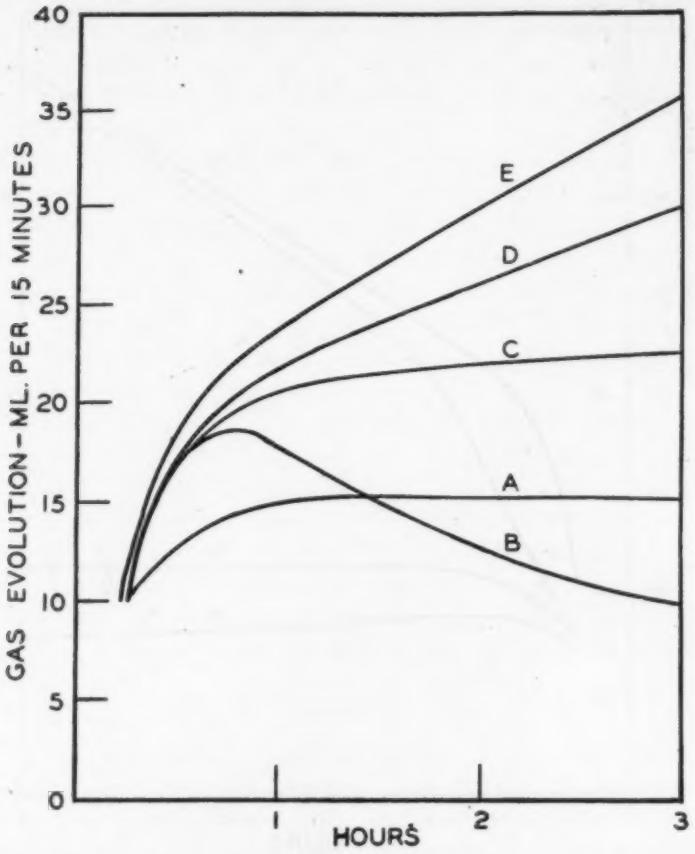


Fig. 2. The effect of phosphate, sulfate, magnesium, and potassium on fermentation rate. Temp. 30°C, total vol. 20 ml, yeast 0.6 g, sugar (sucrose) 2 g.

A. "Complete" medium minus $NaH_2PO_4.H_2O$.
B. "Complete" medium minus $MgSO_4.7H_2O$, but plus $Na_2SO_4$ 0.04 g.
C. "Complete" medium minus $MgSO_4.7H_2O$, but plus magnesium lactate 0.04 g.
D. "Complete" medium minus K Cl.
E. "Complete" medium; $NaH_2PO_4.H_2O$ 0.06 g, $MgSO_4.7H_2O$ 0.04 g, K Cl 0.016 g, asparagine 0.2 g, thiamine 0.00008 g, pyridoxine 0.00008 g, nicotinic acid 0.0008 g, and sodium citrate buffer (M/3) pH 5.5 2.4 ml.

separate test showed that a mixture of the two acted in all respects like magnesium sulfate in tests of the present type.

The influence of the single factors, when added to a simple sugar-water mixture, is shown in Figure 3. Separately, they are without effect—only in the "complete" mixture is their importance apparent.

*Influence of Ammonia or Amino Nitrogen.* If no available nitrogen is present in the fermentation mixture, the rate of fermentation does not attain a level much over 15 ml and no acceleration is apparent (see Figure 4). The addition of asparagine to the sugar-water mixture is without significant effect. Ammonium salts have essentially the

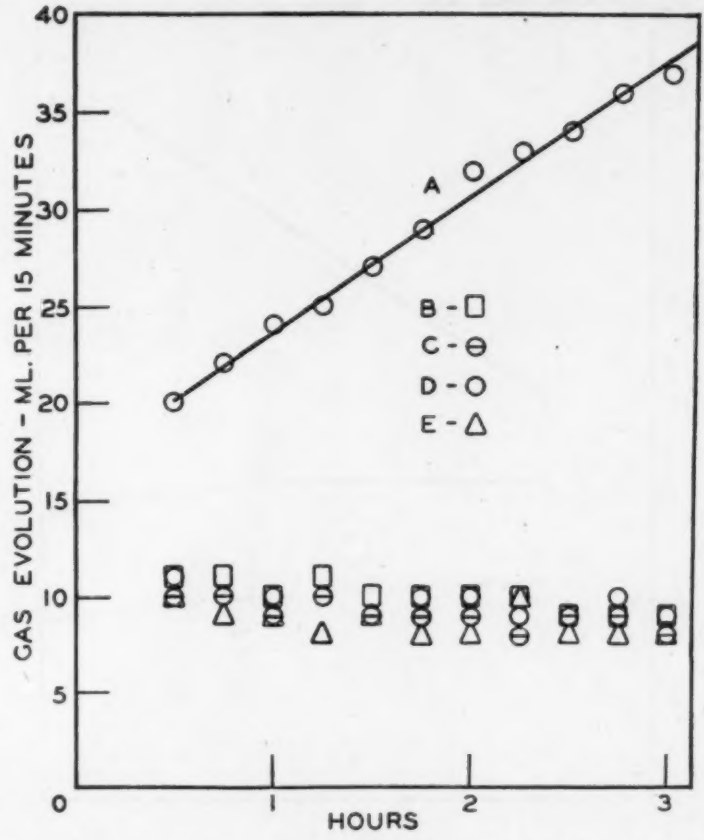


Fig. 3. Effect of potassium chloride, sodium phosphate, and magnesium sulfate on fermentation rate. Temp. 30°C, total vol. 20 ml, yeast 0.6 g, sugar (sucrose) 2 g.

A. "Complete" medium; $NaH_2PO_4.H_2O$ 0.06 g, $MgSO_4.7H_2O$ 0.04 g, K Cl 0.016 g, asparagine 0.2 g, thiamine 0.00008 g, pyridoxine 0.00008 g, nicotinic acid 0.0008 g, and sodium citrate buffer (M/3) pH 5.5 2.4 ml.
B. No addition except $NaH_2PO_4.H_2O$ 0.06 g.
C. No addition except $MgSO_4.7H_2O$ 0.04 g.
D. No addition except K Cl 0.016 g.
E. No addition.

same influence as asparagine and also certain other amino compounds, such as amino acids (Schultz *et al*, 1939a).

Because amino nitrogen is the factor most prominently lacking in white flour from the standpoint of maximum fermentation, a series of tests was made with suboptimal additions of asparagine. The results

are shown in Figure 5. With suboptimal amounts of asparagine the rate of fermentation tends to rise for a period and then level off.

*Influence of the Vitamins.* If thiamine is omitted from the "complete" mixture, fermentation proceeds at a low level and shows a relatively slight acceleration (see Figure 6). Pyridoxine omission

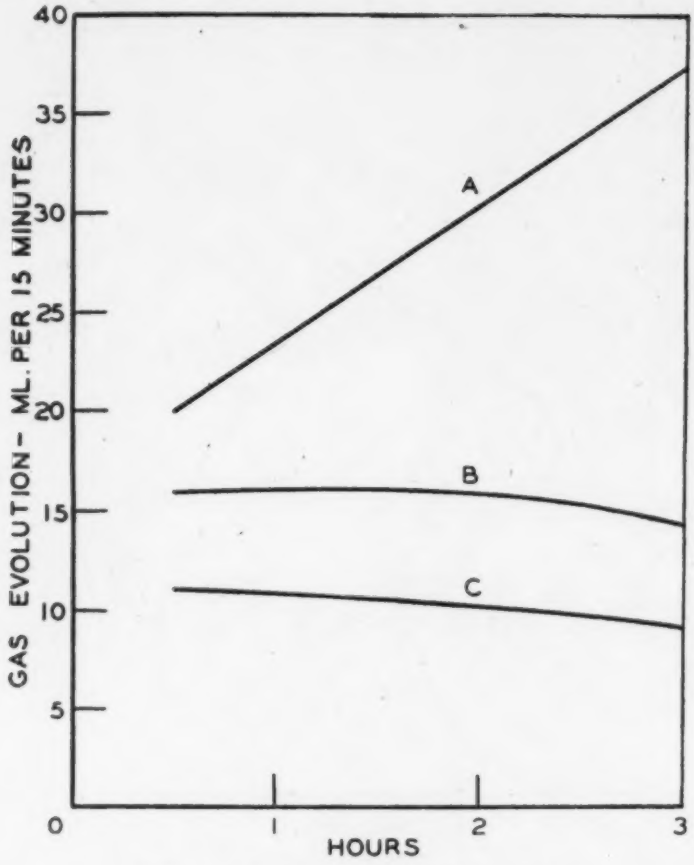


Fig. 4. Influence of asparagine on fermentation.

Temp. 30°C, total vol. 20 ml, yeast 0.6 g, sucrose (sugar) 2 g.

A. "Complete" medium; $NaH_2PO_4.H_2O$ 0.06 g, $MgSO_4.7H_2O$ 0.04 g, K Cl 0.016 g, asparagine 0.2 g, thiamine 0.00008 g, pyridoxine 0.00008 g, nicotinic acid 0.0008 g, and sodium citrate buffer (M/3) pH 5.5 2.4 ml.
B. "Complete" medium minus asparagine.
C. No addition except asparagine 0.2 g.

causes only a slight recession from the maximal rates. The addition of either thiamine or pyridoxine to the sugar-water mixture is without effect. Although nicotinic acid forms part of the "complete" mixture, the effect of its omission, if any, is so slight as to be beyond the sensitivity of the present experimental procedure.

## Discussion

It is apparent from the foregoing that the "complete" synthetic mixture may contain all of the nonsugar chemical factors or their equivalents that control the rate of fermentation in the dough. Each of the factors employed has been used in fermentation media described

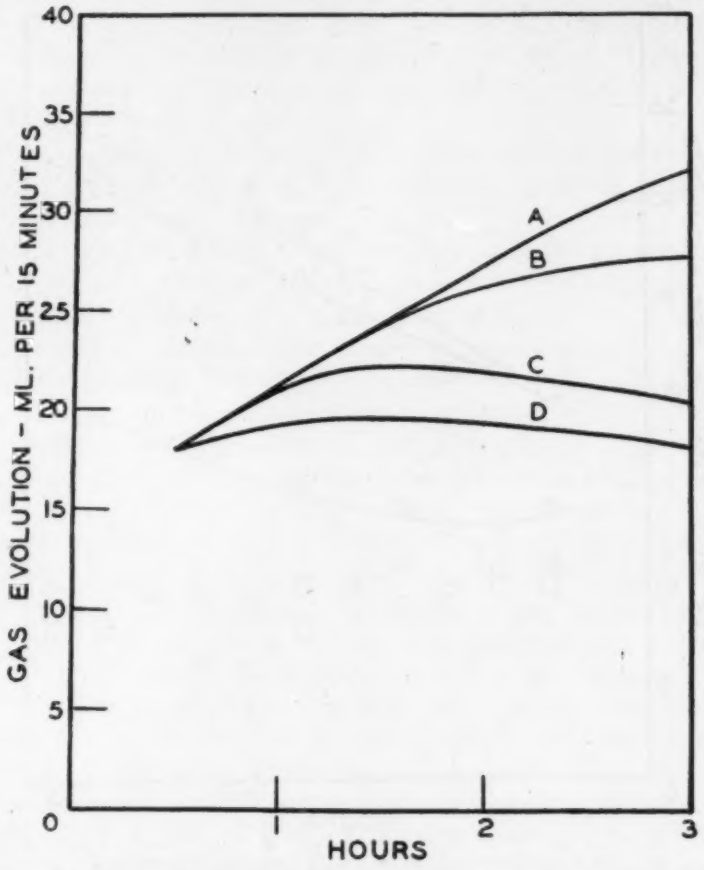


Fig. 5. Influence of concentration of asparagine on fermentation rate in "complete" medium.

Temp. 30°C. total vol. 20 ml. yeast 0.6 g. sugar (sucrose) 2 g. $NaH_2PO_4.H_2O$ 0.06 g. $MgSO_4.7H_2O$ 0.04 g. K Cl 0.016 g. thiamine 0.00008 g. pyridoxine 0.00008 g. nicotinic acid 0.0008 g. and sodium citrate buffer (M/3) pH 5.5 2.4 ml.

A. 80 mg asparagine.
B. 40 mg asparagine.
C. 20 mg asparagine.
D. 10 mg asparagine.

in the literature at one time or another. Except in the yeast fermentation method of Schultz *et al* (1942), the factors have not been combined in a synthetic mixture to promote rapid or maximal fermentation.

Phosphate has long been known to be concerned with the internal mechanism of alcoholic fermentation (Harden, 1932). It has been

employed in yeast nutrient solutions from early times (Jörgensen, 1925). Phosphate enters into the carboxylase enzyme system in the form of cocarboxylase which is diphosphothiamine (Lohmann and Schuster, 1937). It is common practice to add phosphates to sugar-rich materials, such as molasses, prior to their fermentation. Larmour

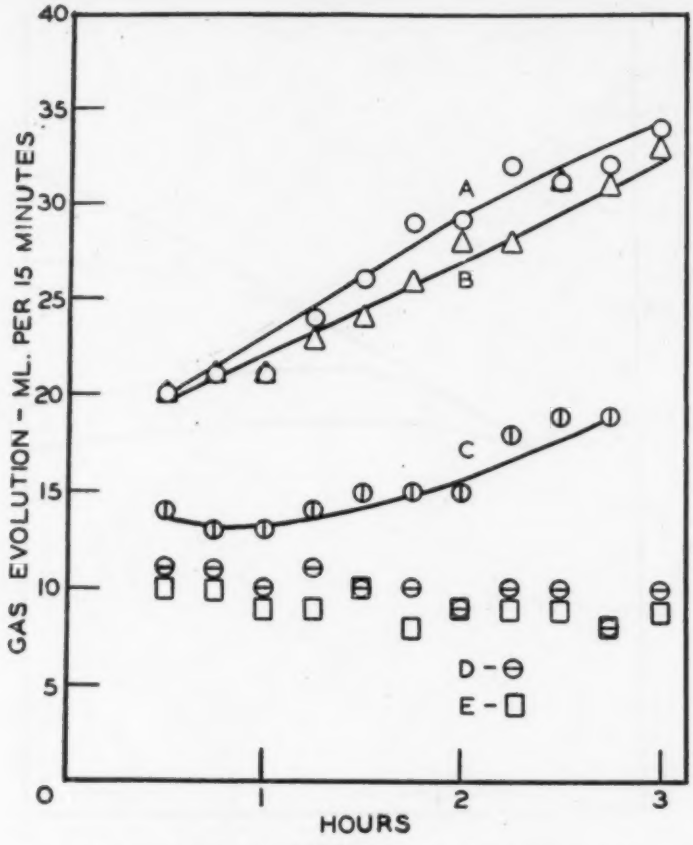


Fig. 6. Influence of thiamine and pyridoxine on fermentation.

Temp. 30°C, total vol. 20 ml, yeast 0.6 g, sucrose (sugar) 2 g.

A. "Complete" medium; $Na_2PO_4.H_2O$ 0.06 g, $MgSO_4.7H_2O$ 0.04 g, K Cl 0.016 g, asparagine 0.2 g, thiamine 0.00008 g, pyridoxine 0.00008 g, nicotinic acid 0.0008 g, and sodium citrate buffer (M/3) pH 5.5 2.4 ml.
B. "Complete" medium minus pyridoxine.
C. "Complete" medium minus thiamine.
D. No addition except thiamine 0.00008 g.
E. No addition except pyridoxine 0.00008 g.

and Bergsteinsson (1936), however, state that the phosphate ion has little, if any, specific effect on gas production. Their experiments were made with fermenting doughs, and as Swanson (1912) has shown, there is a significant quantity of water-extractable phosphorus in patent flour. Therefore, it must be assumed that if phosphate does

play a role in yeast fermentation in the dough, the flour used by Larmour and Bergsteinsson (1936) contained an excess. The nature of the soluble phosphate in flour is not exactly known, but Swanson (1912) proposes that at least a part is monopotassium phosphate. A good part of the phosphorus of wheat exists in organic combination, either as phytates or phospholipids (Bailey, 1944). Widdowson (1941) has reported that 85% of the phytic acid originally contained in white flour is destroyed in the preparation of yeast bread. It is possible that destruction of phytic acid coincides with the liberation of soluble phosphate or phosphoric acid.

The proportion of the phosphate of flour which is separated from the organic moieties by hydrolysis during the doughing process is at present undetermined.

Sulfur, like phosphorus, is an important constituent of cell protoplasm. It has not heretofore been reported to be directly associated with alcoholic fermentation. In the form of sulfate it is available to the cell for the synthesis of the sulfur-containing amino acids, cystine and methionine, and the tripeptide glutathione. Magnesium sulfate is a constituent of the medium of both Hansen (Jörgensen, 1925) and Slator (1906). Although there is sulfur in the ash of white flour (Bailey, 1944), there is no evidence concerning the form of its compounds. Neither are there any data on the proportion which is water soluble. It seems probable from the data of the present report that sulfate in adequate amount is present in white flour.

Magnesium also has been employed in yeast nutrient solutions since early times (Jörgensen, 1925). It is concerned with the internal mechanism of alcoholic fermentation (Kossel, 1942), (Green, Herbert, and Subrahmanyan, 1941). Harding and Dysterheft (1927) found 0.0613% of magnesium in clear flour. Here, as with phosphorus and sulfate, the nature of the soluble magnesium compounds is not known.

The exact role of potassium in fermentation is not known, but its importance in accelerated fermentation can be judged from Figures 5 and 6. As with phosphorus and magnesium, there is evidence that flour contains significant quantities of potassium and, hence, conceivably could make available adequate amounts for the nutrition of the yeast. Lasnitzki and Szörényi (1935) observed that potassium increased the rate of fermentation of bakers' yeast. Whether all flours contain available phosphate, sulfate, magnesium, and potassium in sufficient amount to prevent these nutrients from ever becoming a limiting factor in dough fermentation is not known.

Willard and Swanson (1913) found that the rate of fermentation of doughs was increased by the addition of peptones, certain amino acids, and ammonium salts. Ammonium chloride gave a marked

increase, but ammonium phosphate (dibasic) was reported to be without action. These authors also found that an extract of bran or wheat scourings had a stimulatory effect on dough fermentation.

In 1915, Kohman, Hoffman, and Blake (1915) obtained a patent covering the addition of ammonium salts to the dough for the purpose of enhancing fermentation activity. Although ammonium salts or assimilable amino-nitrogen compounds had been employed for yeast growth in nutrient solutions earlier, it was not widely recognized until recently that such substances are fermentation accelerators. Zeller (1926) observed a stimulation of fermentation due to a large number of ammonium salts. Euler and Larsson (1934) observed that the activity of one of their Factor Z preparations was higher when it contained ammonium ions. When tested separately, ammonium sulfate gave them some stimulation, but much less than their concentrate of Factor Z. Larmour and Bergsteinsson (1936) observed that ammonium salts exert a specific stimulation in dough fermentation, particularly in the latter stages, *i.e.*, after the first hour or two.

Sandstedt and Blish (1938) observed that flours differed widely in the third hour or "proof time" fermentation rate, when tested in the following formula: 10 g of flour, 0.4 g of maltose, 10 ml of $H_2O$, and 0.3 g of yeast. They ascribed the differences to a variation in factor M content, factor M being a specific catalyst for maltose fermentation. Schultz *et al* (1939a) suggested that the difference between flours might be due to their content of amino nitrogen. They showed that the addition of various amino acids increased the third-hour fermentation rate to a significant extent, and tended to make the rates of different flours alike. Ofelt and Sandstedt (1940) found that the third-hour proof rate of a series of flours increased in almost linear relationship with increasing protein content. Hydrolyzed gluten was added to a number of samples of flour of widely different protein content and this brought all flours to a nearly uniform rate of third-hour gas production. Ammonium salts accomplish the same thing, but they found that *l*-asparagine did not quite succeed in bringing all rates to the same level.

Yeast is capable of utilizing nitrogen from other sources besides ammonium salts (Thorne, 1933). The amide nitrogen of asparagine is readily available to yeast, probably because yeast contains an asparaginase that splits off the ammonia from the amide group (Grassmann and Mayr, 1933).

As Larmour and Bergsteinsson (1936) have observed, the effect of ammonium salts added to the dough appears in the latter stages of fermentation. The tests with suboptimal amounts of asparagine (described earlier) indicate that this may be explained by the parallel

paths of fermentation initially, which diverge when available nitrogen becomes the limiting factor.

Euler and Swartz (1924) are responsible for initiating the intensive study of an organic substance, occurring widely in nature, that is capable of causing a marked stimulation of alcoholic fermentation by living yeast. They called this substance Factor Z. Euler and his co-workers devoted much work to the study of the nature of the elusive substance (Euler and Larsson, 1934). Schultz *et al* (1937, 1937a) observed that vitamin $B_1$, later designated thiamine, had the properties of Factor Z. When the activity of the pyrimidine moiety of the thiamine molecule was shown (Schultz *et al*, 1937a), the identity of Factor Z and thiamine (or the specific amino pyrimidine) became even more certain. Euler's school had noted that alkaline hydrolysis did not destroy Factor Z activity, and for this reason they did not associate vitamin $B_1$ and Z factor, since vitamin $B_1$ activity was known to be alkali labile. The amino pyrimidine produced by hydrolysis of thiamine retains the fermentation accelerating activity, although inactive as a vitamin.

Thiamine is always present in bread flour. Although a large proportion of the thiamine of the wheat kernel is removed in the course of the milling process which produces white flour (Schultz *et al*, 1939b), the amount retained is sufficient to exert maximal fermentation acceleration in most cases (Schultz *et al*, 1939a). In the latter publication these authors describe one flour (first middlings) so low in thiamine that addition of the vitamin caused a significant increase in the third-hour fermentation rate. Although thiamine deficiency does not appear to be a normal occurrence in bread manufacture, it must be recognized that thiamine is an essential factor in the mechanism of alcoholic fermentation in the dough.

Pyridoxine (vitamin $B_6$) has a much smaller effect upon fermentation (Schultz *et al*, 1939). Like thiamine it is present in white flour in amounts adequate to promote maximal fermentation (Atkin, Schultz, Williams, and Frey, 1943).

From the whole of the present discussion it will become apparent that any single one of the factors essential for fermentation might be present in a flour in suboptimal amount for maximal fermentation with 3% yeast, but such deficiency would not be noticed with a lower percentage of yeast, or in a dough which has not been supplied with optimal amounts of other necessary factors, *e.g.*, amino nitrogen.

## Summary

A solution containing potassium-, magnesium-, phosphate-, and sulfate-ions, thiamine, pyridoxine, nicotinic acid, a source of yeast-

available nitrogen, sugar, and a buffer is found to support a rate of fermentation equal to that in a flour suspension fortified with an excess of sugar and available nitrogen. The specific influence of these factors, all of which are constituents of flour, is described.

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## THE DISTRIBUTION OF THIAMINE AND RIBOFLAVIN IN WHEAT GRAINS

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Information regarding the distribution of thiamine and riboflavin is found in recent milling data (Andrews, 1943; Newman, 1942; Schultz, Atkin, and Frey, 1939; Sherwood, Nordgren, and Andrews, 1941; Shetlar and Lyman, 1941) where it is shown that low extraction flours consisting principally of the endosperm, without the aleurone layer, are low in thiamine and riboflavin. When the extraction is extended beyond about 75%, fractions are obtained with much higher thiamine and riboflavin contents. In the case of riboflavin, this increase continues until all of the grain is included, but in the case of thiamine the last fraction, which represents the bran fraction, is lower in thiamine content than those fractions which immediately precede it. These data are interpreted to mean that the outer portions and the germ of the wheat grain are richer in thiamine and riboflavin than the inner endosperm, with the exception that the outer bran coats are relatively low in thiamine.

The interpretations based upon milling data have been corroborated and extended by various dissection studies. It has been shown that

the embryo half of the wheat grain is much richer in thiamine than the brush half (Andrews, 1943; Jackson and Whiteside, 1942; and Ward, 1943). It has also been shown that the cells immediately surrounding the germ are extremely rich in thiamine (Newman, 1942). Recently the distribution of thiamine in the embryo [1] has been studied by Hinton (1942 and 1944) and Ward (1943) who dissected out the various parts and analyzed them. Ward found that the scutellum and its epithelium contained six to eight times as much thiamine as the primary root, cotyledon, primary leaves, etc. Hinton (1944) found that on an average the scutellum contains nearly 20 times as much thiamine per gram dry weight as the other parts of the embryo.

Similar dissection studies for riboflavin content have been very limited in number. In the paper by Hinton (1942) are cited a few data obtained by Barton-Wright. These data indicate that the various parts of the embryo are nearly equal in riboflavin content. Certainly the scutellum does not appear to be especially rich in this vitamin. Bailey (1944) has recently reviewed the subject of the vitamin content of wheat and wheat products; hence further review will not be presented here.

## Method

We recently described a simple method for locating thiamine and riboflavin in wheat kernels (Somers and Coolidge, 1945). The method consists of cutting the wheat grain to produce a smooth surface, which is then treated with an alkaline solution of potassium ferricyanide. This treated surface fluoresces when illuminated with a mercury vapor lamp provided with a Corning No. 5970 (597) [2] filter. The light transmitted by this filter excites the thiochrome fluorescence, which is viewed through a filter combination consisting of Corning filters Nos. 3385 (338) [2] and 4308 (428).[2] To obtain the fluorescence which we think is characteristic for riboflavin or some closely related derivative, Corning filters Nos. 5113 (511) [2] and 3389 (038) [2] are used on the mercury vapor lamp, and No. 3482 (349) [2] is used for viewing the fluorescence. By using these and other filter combinations and a photomicrographic camera with microtessar lenses, we have photographed several grains. Some of these photographs are shown in Figures 1 to 5.

This method will not distinguish between thiamine and cocarboxylase. This should be kept in mind because we make no attempt in the

[1] It is necessary to use some caution in terminology when speaking of the various tissues of the wheat grain since there is a tendency in the technical literature to use terms which do not correspond to the original anatomical definition. Thus, the term embryo, as used in this paper, includes the scutellum, with its epithelium, as well as other parts of the embryo. This does not correspond with terms used by Hinton (1942 and 1944) and Ward (1943). Likewise, according to anatomical definition, the aleurone layer is part of the endosperm.

[2] The old Corning code numbers for the various filters are given in parentheses.

discussion below to distinguish between these two; instead, we speak only of thiamine. However, it appears that wheat does not contain significant amounts of cocarboxylase (Obermeyer, Fulmer, and Young, 1944).

## Results and Discussion

Figure 1 shows photographs of a series of strips of filter paper treated in various ways and photographed with the filter combinations described above. The strips in Figure 1*A* were photographed using Eastman Contrast Process Ortho film, were developed in D76 developer, and were printed on a high contrast paper. In these respects they are comparable with Figures 2, 3, 4, and 5*A*. It will be noted (lower series of strips) that the filter combination for thiochrome

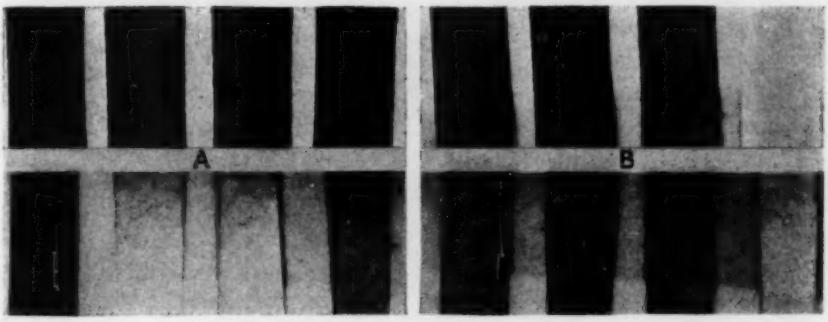


Fig. 1. Photographs of treated filter paper strips. (*A*) Photographs obtained by using filters selective for thiochrome fluorescence, *i.e.* Corning No. 5970 (597) on the ultraviolet lamp and Corning Nos. 3385 (338) and 4308 (428) on the camera. *Top row: left to right*, control, thiamine, cocarboxylase, riboflavin. *Bottom row:* same as top row, except that all the strips have been treated with an alkaline ferricyanide solution. (*B*) Similar to *A*, except that filters selective for riboflavin fluorescence were used, *i.e.* Corning Nos. 5113 (511) and 3389 (038) on the lamp and Corning No. 3482 (349) on the camera. The vitamins and cocarboxylase were applied to all of the filter paper strips except the controls in amounts equal to approximately 0.01 ml of a millimolar solution per square centimeter; hence the concentrations are comparable for all of the strips. Those that were treated with an alkaline ferricyanide solution were all treated alike, after they had dried in the dark, with a mixture of 5 parts of 5% sodium hydroxide and 1 part of 0.5% potassium ferricyanide. They were then dried in the dark before being photographed.

fluorescence distinguishes sharply between oxidized thiamine and cocarboxylase, on the one hand, and riboflavin and the control, on the other hand. This filter combination, with this type of film, does record some of the riboflavin fluorescence, but, in view of the fact that the riboflavin content of wheat is less than the thiamine content (see Bailey, 1944, for a summary), this interference is probably not serious. However, by using another filter combination, *i.e.* Corning filter No. 5840 (584) on the lamp and Nos. 5543 (554) and 3389 (038) on the camera, it is possible to eliminate the interference due to riboflavin. With this filter combination, however, there appears a weak fluorescence in the aleurone layer of untreated grains which was

considered more serious than the possible slight interference due to riboflavin with the other filter combination.

The filter paper strips in Figure 1*B* are the same as those in Figure 1*A*. The only difference is in the filters and film used. For Figure 1*B* the filters were selective for riboflavin fluorescence, and the film was Eastman Panatomic X. Somewhat longer exposures were required with this filter combination than with the combination used for

Fig. 2. Two wheat grains cut longitudinally through the crease and photographed using filters selective for thiochrome fluorescence (*cf.* Figure 1*A*). The grain on the left has not been treated with an alkaline ferricyanide solution; the one on the right has been treated. 15×

thiochrome fluorescence, and Eastman Contrast Process Ortho film could not be used. The fluorescence produced by riboflavin was apparently not affected by the oxidation with alkaline ferricyanide, in so far as our method is concerned. As is evident from the photographs, this fluorescence can be sharply distinguished from the fluorescence of thiochrome and oxidized cocarboxylase.

The light background in Figures 1*A* and 1*B* was obtained by

mounting the filter paper strips in front of modeling clay. This clay fluoresces; some colors fluoresce more brightly than others. This difference in fluorescence can be seen in Figures 1*A* and 1*B*. The darker background was produced by a grey- and the lighter background by an ivory-colored clay.

Figures 2, 3, 4, and 5*A* are photographs of various cut surfaces of wheat grains, and they show the distribution of the fluorescence which is seen by using the filter combination for thiochrome fluorescence. As is evident from Figure 2, the untreated grains show no fluorescence. Figures 2, 3, 4, and 5*A* indicate that the thiamine in the endosperm is located principally in the aleurone layer; in the cells surrounding the bottom of the crease, or groove, of the grain; and in the cells adjacent to the embryo. The thiamine appears to be principally in

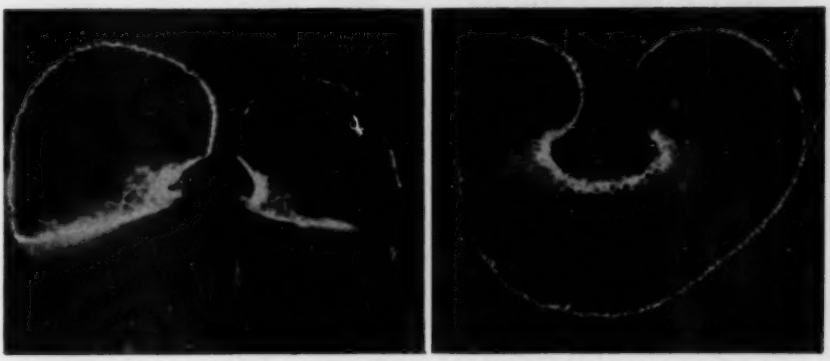

Fig. 3. Photograph of cross-section surfaces of two wheat grains using filters selective for thiochrome fluorescence (*cf.* Figure 1*A*). The grain on the left has been cut through the embryo. The grain on the right has been cut through the endosperm near the brush end of the grain. Both grains were treated with an alkaline ferricyanide solution. 21 ×

the cell walls, and even the cell walls of the inner endosperm cells appear to contain some of this vitamin. Figure 4 indicates that the outermost wall of the aleurone cells contains much less thiamine than the other walls.

Figures 2 to 5*A* also show the distribution of the thiochrome fluorescence in the embryo. There appears to be a region of particularly high concentration that either coincides with the epithelium of the scutellum or is adjacent to it. The remainder of the scutellum also contains considerable thiamine. In some grains we have observed a greater fluorescence in the scutellum than these figures show. There is some variation from kernel to kernel. Some such variation can be observed by comparing Figure 2 with Figure 5*A*. In all cases, all parts of the embryo, exclusive of the scutellum and its epithelium, appear to contain very little thiamine. Occasionally, however, concentrated bands

of thiamine do appear in the epidermal cells of either the coleoptile or one of the primary leaves. One such band is observed in Figure 2.

A comparison of Figures 5*A* and 5*B*, which are of the same grain of wheat, indicates that riboflavin and thiamine are distributed differently in the wheat grain. Riboflavin is more uniformly distributed in the embryo. The aleurone layer apparently contains considerable amounts of the vitamin, and the bran coats also appear to contain some. The inner cells of the endosperm appear to contain very little riboflavin.

The fact that the thiamine in wheat appears to be located in the

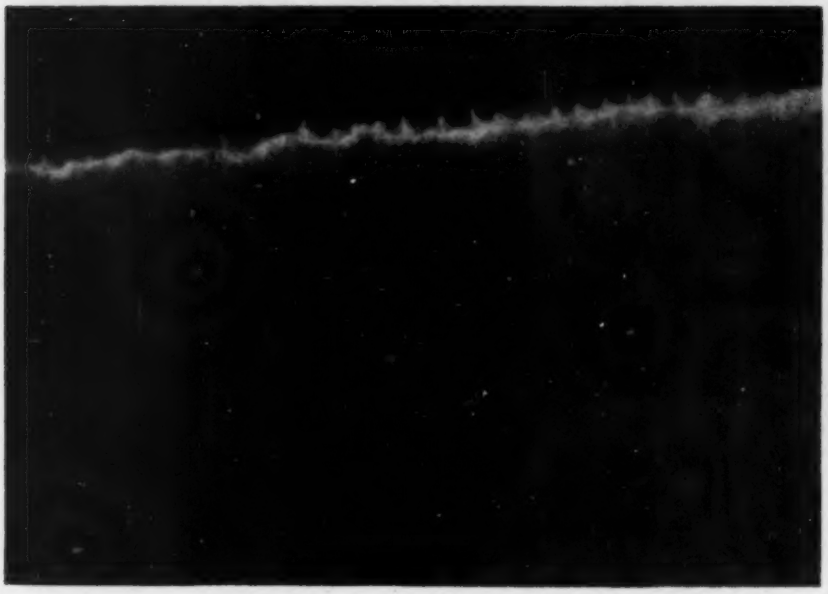

FIG. 4. A portion of a longitudinal-section surface through the aleurone layer of a wheat grain showing the distribution of thiochrome fluorescence. The aleurone layer is the layer of rectangular cells, the walls of which fluoresce brightly. 101 ×

walls of cells in which it occurs is rather surprising. We cannot at present say that the cell walls, as such, contain thiamine. It is conceivable that the surface layers of protoplasm contain the thiamine and that these layers are so closely pressed against the cell walls as to be indistinguishable from them by our technique. If the cell walls do not contain thiamine one might expect to see dark lines between the fluorescent regions of adjacent cells. Even at the highest magnification (*cf.* Figure 4) such a condition is not observed. There are several possible interpretations for our failure to obtain such a picture. First, the cell walls may actually contain thiamine, or the thiamine may be washed into them by the small amount of moisture used to treat the

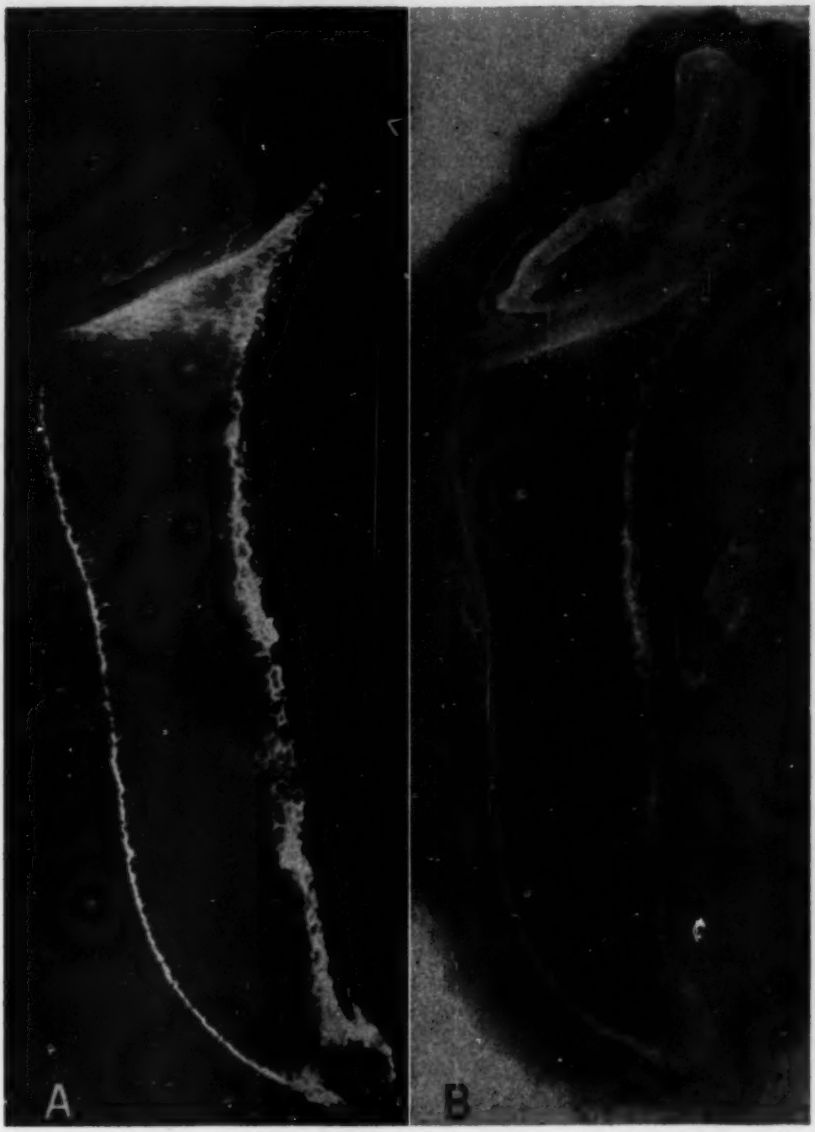


Fig. 5. Longitudinal-section surface through the crease of a single wheat grain. The photograph on the left (*A*) was taken using the filters selective for thiochrome fluorescence (*cf.* Figure 1*A*), and the photograph on the right (*B*) was taken using the filters selective for riboflavin fluorescence (*cf.* Figure 1*B*). 24×

grain. In either case, one would not expect to find dark lines between adjacent cells. Second, our technique may not be sufficiently refined to show such fine details, if they do exist. More research will be required to decide the question.

So far, this technique has been applied only to wheat. It is so simple that it should prove useful for similar studies with other cereals. It also may be possible to apply it to other materials which contain less thiamine and riboflavin than the cereals.

## Summary

Photographs of the fluorescence produced by illuminating cut wheat grains following treatment with an alkaline solution of potassium ferricyanide are presented. These indicate that the thiamine of the wheat grain is located principally in the aleurone layer, in the endosperm cells adjoining the aleurone cells at the base of the crease, in the scutellum of the embryo (including its epithelium), and in the endosperm adjoining the scutellum. These photographs indicate that all parts of the embryo contain approximately equal amounts of riboflavin. The embryo and the aleurone layer appear to be regions of the grain containing the greatest concentration of riboflavin, although the bran coats appear to contain some of this vitamin.

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# EFFECT OF KILNING ON THE AMYLOLYTIC ACTIVITY OF BARLEY MALTS

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Green malt, when subjected to certain controlled kilning conditions, undergoes far-reaching physical and biochemical changes. When preceded by germinating conditions portending maximum enzyme formation, the extent of these alterations is usually characteristic of the barley variety and its geographical origin.

Loss in enzymatic activity has long been associated with the dehydration of the green malt. Kopecky (1936) concluded that the loss in diastatic power during kilning is directly proportional to the initial concentration. He contended, moreover, when comparing green malts, that those showing the highest diastatic activity did not always show highest in the kilned malt.

Delbrück (1908) asserts that even under the most favorable conditions only 85% of the original diastase of the green malt remains in the finished product; that in commercial malting the diastase loss is so great during kilning that a specified weight of green malt has the same potency for the distiller as the same weight of kilned malt.

Foth (1929) maintains that malts intended for conversion purposes in the distillery retain only 80% of their initial diastase when kiln dried at 50°C (122°F). He cites the work of Kjeldahl (1879) which purported to show that green malts dried in the air at 30°C (86°F) retained only 85% of their original diastatic power.

In the commercial production of diastatic malts it has been found, however, that malts can be prepared which show considerable increase in diastatic activity over the green malt. This has been accomplished by allowing the moisture content to remain between 48% and 52% and the temperature at approximately 40°C (105°F) during the first 24 hours of the kilning process, the passage of air through the mass being greatly retarded.

This primary kilning stage cannot be compared to an extra day of flooring because (1) the amylolytic activity increases but a relatively few units from the sixth to the seventh day (on the floor), and (2) there is an insignificant decrease in total dry matter in malts dried in this manner over those dried rapidly at higher temperatures by large volumes of air.

By applying such treatment, the alpha-amylase activity is increased

about 32% over its value in the green malt and the beta-amylase shows an increase of about 25 saccharifying units.

In the present study, it was undertaken to demonstrate the progressive increase of the amylolytic components during primary kilning and final drying. Additional experiments were conducted in which the effect on amylase of the conventional procedure of rapid drying was compared with one employing initial slow drying. As sulfur dioxide was allowed to pass through the green malts when loaded on the kiln, the investigation was extended to consider its effect on the diastatic power.

## Materials and Methods

Rough-awned barley types from South Dakota (1942 and 1943 crop), North Dakota, and Canada, in as pure lots as were commercially available, were selected for these tests. All malts were germinated in

TABLE I

Effect of Particle Size on Diastatic Activity of Green Malt

| Treatment | Moisture | Diastatic power | α-Amylase (activity) | β-Amylase (activity) |
|---|---|---|---|---|
| | % | °L | *mg maltose* | *mg maltose* |
| Water extracted | 60.6 | 196 | 35.3 | 749 |
| Water extracted and sample forced through No. 30 sieve | 60.6 | 195 | 35.3 | 745 |
| $Ca(OAc)_2$ extracted | 60.6 | 202 | 61.2 | 747 |
| $Ca(OAc)_2$ extracted and sample forced through No. 30 sieve | 60.6 | 203 | 61.2 | 751 |

quantities of 1,500 bushels or larger and were kiln dried in like quantity unless otherwise stated.

The methods used for the evaluation of the alpha- and beta-amylase activities of malts were those of Sandstedt, Kneen, and Blish (1939) and Kneen and Sandstedt (1941) as modified by Olson, Evans, and Dickson (1944).

After macerating the green malts for 5 minutes in the Waring Blendor with 200 to 300 ml of water, the total volume of water added was made to 500 ml and the whole allowed to extract for 2.5 hours at 20°C before filtering. The alpha- and beta-amylase activities of this filtrate were then determined.

In calculating activity values, a correction factor of 3.3° Lintner (°L) was added to the green malt diastatic power (D.P.) for each percent of sprouts present in the sample. Thus an addition of 10°–12°L was made to the diastatic power originally obtained.

When first associating increased diastatic activity with controlled kilning, it was believed that the evaluation of the green malt, by pre-

paring water extracts by the method outlined, was incomplete, owing to an insufficient comminution on the kernel. To investigate this, different portions of the same green malt were ground in the Waring Blendor with water and with 0.2% calcium acetate solution, and the samples forced quantitatively through a standard U. S. number 30 sieve. Replicate grindings without sieving served as controls. The results, taken from the analysis of a five-day-old piece, appear in Table I.

The decrease in particle size caused no change in either the alpha- or beta-amylase activity.

Extractions of green, partially dried, and kiln-dried malts were made with distilled water and with 0.2% calcium acetate solution, respectively. As illustrated in Tables I and II, the presence of this salt in solution activated or released approximately an additional 70% of alpha-amylase in each case.

TABLE II

EFFECT OF EXTRACTION OF MALT WITH 0.2% CALCIUM ACETATE SOLUTION ON AMYLASE ACTIVITY

| Extracting medium | α-Amylase (activity) | β-Amylase (activity) | pH | Titratable acidity [1] |
|---|---|---|---|---|
| | *mg maltose* | *mg maltose* | | *ml* |
| Water | 48.7 | 807 | 5.77 | 4.00 |
| $Ca(OAc)_2$ | 82.0 | 801 | 5.73 | 5.70 |

[1] Expressed as ml of 0.10 *N* NaOH required to bring 50 ml (equivalent to 2.5 g of malt) of a cold water extract to pH 8.5.

As the gain in amylolytic activity due to extraction with calcium acetate solution is but a relative one, only those activities obtained by water extractions will be referred to in the following discussion.

**Effect of Heat Treatment of Green Malt on Activity of the Amylases**

The effect of heat on the amylolytic enzymes of green malt, while maintaining constant moisture, can be demonstrated in the laboratory at 45°C (113°F). At this temperature, however, a considerable decrease in beta-amylase activity has been noted in certain malts. Table III illustrates the increase in alpha-amylase activity achieved by this treatment, and further shows that this increase is maintained in the finished malt. The increase in titratable acidity in both the heated green malt and that subsequently dried is worthy of mention.

The stimulating effect of heat on the activity of the alpha-amylase prompted repetition of the experiment on a plant scale.

Four lots of barley were germinated and sampled on the fifth and sixth day. The increase in diastatic power by the additional day of

TABLE III

EFFECT OF HEATING GREEN MALTS AT 45°–49°C (113°–120°F) AT ISOMOISTURE LEVELS FOR 1–2 HOURS ON AMYLASE ACTIVITY, pH, AND TITRATABLE ACIDITY

| Sample | LABORATORY SCALE GREEN MALT | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | UNHEATED MALT | | | | | | HEATED MALT | | | | | |
| | Moisture content | Diastatic power | α-Amylase (activity) | β-Amylase (activity) | pH | Titratable acidity | Moisture content | Diastatic power | α-Amylase (activity) | β-Amylase (activity) | pH | Titratable acidity |
| | % | °L | *mg maltose* | *mg maltose* | | *ml* | % | °L | *mg maltose* | *mg maltose* | | *ml* |
| A | 56.1 | 195 | 41.5 | 738 | 5.32 | 4.85 | 56.3 | 197 | 57.0 | 730 | 5.97 | 5.35 |
| B | 51.8 | 153 | 29.8 | 582 | 5.40 | 3.70 | 52.4 | 155 | 37.9 | 584 | 5.54 | 4.10 |
| C | 54.5 | 178 | 38.5 | 673 | 5.38 | 3.90 | 51.2 | 184 | 41.8 | 694 | 5.72 | 4.20 |
| | THE ABOVE GREEN MALTS "RAPID DRIED" AT 43°–46°C (110°–115°F) | | | | | | | | | | | |
| | UNHEATED MALT | | | | | | HEATED MALT | | | | | |
| A | 6.4 | 171 | 44.7 | 640 | 5.88 | 3.25 | 6.9 | 174 | 59.2 | 635 | 5.55 | 3.95 |
| B | 8.1 | 142 | 36.4 | 530 | 6.05 | 1.85 | 8.2 | 144 | 48.0 | 525 | 6.14 | 4.10 |
| C | 8.1 | 162 | 50.0 | 598 | 5.19 | 2.60 | 8.7 | 168 | 51.8 | 620 | 5.50 | 3.10 |

germination averaged 17°L (Table IV), which figure agrees well with an increase of 15°L previously noted by the writer in some three hundred similar observations.

The upper kiln or primary drying phase replaced the laboratory oven treatment. The green malts were loaded on the kiln and held at 40°C (105°F) for 20 hours, while the moisture content was maintained above 45%. This was accomplished by retarding the passage of heated air through the bed of malt.

As indicated in Table IV, the increase of alpha-amylase activity paralleled that observed in the laboratory, while the beta-amylase also showed considerable increase.

It is apparent that the major portion of the increase in diastatic power from the fifth to sixth day was due to an increase in beta-amylase activity. Unpublished investigations on the increase in activity from the sixth to the seventh day reveal it to be less than half that from the fifth to the sixth day.

In following the course of development of enzymatic activity of malt on the upper kiln, it may be noted that the average increase in alpha-amylase activity during the 20-hour stay is almost twice as great as the increase from the fifth to the sixth day. An additional gain of alpha-amylase activity occurred during the final kilning period. There was an over-all average increase of over 30% in the activity of the alpha-amylase from the time the malt was loaded on the kiln until it was finished. Although showing a small reduction during final kilning, there was likewise an over-all average increase in beta-amylase activity of 26 units.

Two-pound samples of the green malts were taken at the fifth and sixth day respectively and rapidly dried at 43°C (110°F). The data obtained upon analysis of these samples are given in Table IV.

Omission of the upper kiln, or low temperature phase, of the kilning operation eliminated part of the gain in alpha-amylase activity normally experienced during kilning; the beta-amylase activity also showed a loss of 20 beta-amylase units (about 5°L). Moisture contents of these malts, dried rapidly at 43°C (110°F), were much higher than those of dried malts subjected to the high-moisture top kiln phase.

### Effect of Sulfur Dioxide on Diastatic Power

Immediately after loading the upper kiln, sulfur dioxide was applied to all of the green malts that have been followed through the phases of kilning. Previous to loading, the green malt was analyzed for its sulfur dioxide content by the Monier-Williams method as outlined by the Association of Official Agricultural Chemists (1940). Determinations

## TABLE IV

### Effect of Kilning on the Amylolytic Activity of Malt

| Sample | Moisture % | Diastatic power °L | α-Amylase (activity) *mg maltose* | β-Amylase (activity) *mg maltose* | pH | Titratable acidity *ml* |
|---|---|---|---|---|---|---|
| **Five-day Green Malts** | | | | | | |
| 1 | 57.4 | 175 | 39.4 | 661 | 5.39 | 4.25 |
| 2 | 61.6 | 190 | 44.4 | 715 | 5.59 | 4.45 |
| 3 | 58.2 | 177 | 25.2 | 683 | 5.10 | 3.40 |
| 4 | 58.5 | 183 | 25.5 | 670 | 5.30 | 3.30 |
| Average | 58.9 | 179 | 33.6 | 682 | — | — |
| **Six-day Green Malts** | | | | | | |
| 1 | 57.5 | 199 | 41.4 | 754 | 5.33 | 4.90 |
| 2 | 58.6 | 197 | 45.5 | 740 | 5.35 | 5.20 |
| 3 | 55.9 | 192 | 34.8 | 733 | 5.50 | 3.80 |
| 4 | 56.4 | 197 | 37.9 | 750 | 5.50 | 3.60 |
| Average | 57.1 | 196 | 39.9 | 744 | — | — |
| **Six-day Green Malts after Being Held "Green" on Top Kiln at 40°C (104°F) for 20 Hours** | | | | | | |
| 1 | 51.9 | 204 | 52.5 | 761 | — | — |
| 2 | 53.2 | 205 | 63.8 | 755 | — | — |
| 3 | 51.2 | 205 | 39.4 | 781 | 5.62 | 4.20 |
| 4 | 54.1 | 214 | 48.7 | 807 | 5.77 | 4.00 |
| Average | 52.6 | 207 | 50.9 | 775 | — | — |
| **Final Kilned Six-day Malts (43°–46°C) (110°–115°F)** | | | | | | |
| 1 | 6.2 | 203 | 54.2 | 756 | — | — |
| 2 | 6.5 | 203 | 63.5 | 749 | — | — |
| 3 | 6.7 | 206 | 47.4 | 776 | 6.03 | 3.00 |
| 4 | 6.9 | 212 | 47.4 | 799 | 6.02 | 3.00 |
| Average | 6.5 | 207 | 52.6 | 770 | — | — |
| **THE EFFECT OF DRYING GREEN MALTS AT 43°C (110°F) IN 20 HOURS** | | | | | | |
| **Kilned Five-day Green Malts** | | | | | | |
| 1 | 8.6 | 173 | 45.8 | 644 | 5.99 | 3.42 |
| 2 | 7.7 | 173 | 48.7 | 653 | 6.05 | 3.30 |
| 3 | 8.8 | 177 | 34.2 | 674 | 6.13 | 2.30 |
| 4 | 8.5 | 183 | 39.4 | 691 | 6.10 | 2.25 |
| Average | 8.4 | 177 | 42.0 | 665 | — | — |
| **Kilned Six-day Green Malts** | | | | | | |
| 1 | 10.1 | 186 | 50.1 | 695 | 6.20 | 3.35 |
| 2 | 9.3 | 200 | 60.4 | 739 | 6.17 | 3.50 |
| 3 | 9.4 | 197 | 37.1 | 751 | 5.82 | 2.80 |
| 4 | 8.9 | 188 | 41.5 | 708 | 5.67 | 2.70 |
| Average | 9.4 | 193 | 47.3 | 724 | — | — |

were also conducted on the "sulfured" green and dried malts. Table V shows the sulfur dioxide absorbed by the malt.

To ascertain the role played by sulfur dioxide in the increase in diastatic power during kilning, an experiment involving 1,500 bushels of green malt was conducted.

TABLE V

ABSORPTION OF SULFUR DIOXIDE BY MALT DURING KILNING

| Treatment | Sulfur dioxide |
|---|---|
| | *ppm* |
| Green malt | negligible |
| "Sulfured" green malt (unground) | 326 |
| "Sulfured" dried malt (unground) | 210 |
| "Sulfured" dried malt (ground) | 409 |

Half of a compartment of malt was loaded on a 1,500 bushel kiln and sulfur dioxide applied. Upon completion of this phase, the remainder of the malt was transferred to the kiln and kilning of the whole conducted in the previously described manner.

As a check against plant operations, two 2-pound samples of the same green malt, taken before and after sulfuring, were rapidly dried at 43°C (110°F), employing high air velocities. The data are shown in Table VI.

TABLE VI

EFFECT OF PLANT SCALE "SULFURING" ON THE AMYLASE ACTIVITY OF MALT

| Phase of operation | "Sulfured" malt | | | "Unsulfured" malt | | |
|---|---|---|---|---|---|---|
| | α-Amylase (activity) | β-Amylase (activity) | Lasche C T value [1] | α-Amylase (activity) | β-Amylase (activity) | Lasche C T value [1] |
| | *mg maltose* | *mg maltose* | *minutes* | *mg maltose* | *mg maltose* | *minutes* |
| Upon completion of germination | 51 | 785 | — | 52 | 788 | — |
| Immediately after "sulfuring" | 53 | 785 | — | — | — | — |
| After 24 hours on top kiln | 71 | 809 | — | 79 | 817 | — |
| At 6.5% moisture | 73 | 800 | — | 67 | 801 | — |
| At 5.0% moisture | 73 | 735 | 8.25 | 65 | 750 | 9.50 |

[1] The Lasche C T value is frequently used to indicate the rate of conversion of starch to dextrins and is determined by observing the time (in minutes) necessary for 2.2 ml of a filtered malt infusion to convert (at 62.5°C) an unbuffered 5% starch solution to the achrodextrin stage.

The values for alpha-amylase activity indicate the usual gain in activity during the first phase of kilning. However, in the instance of the "sulfured" malt, this activity of alpha-amylase was maintained down to the finished product. This does not appear to be the case with the "unsulfured" malt. Upon drying the latter down to 8% moisture,

there was a decrease of 12 units, and upon further drying, there was an additional decrease of two units. Thus, when kilning in the absence of sulfur dioxide, an over-all gain in alpha-amylase, from green to finished malt, of 25% was noted in contrast to a gain of over 40% when sulfur dioxide was applied.

An increase in alpha-amylase activity due to the application of sulfur dioxide was also noted in the case of the rapid-dried malts (Table VII).

TABLE VII

EFFECT OF SULFUR DIOXIDE ON "RAPID-DRIED" MALTS

| Phase of operation | "Sulfured" malt | | | "Unsulfured" malt | | |
|---|---|---|---|---|---|---|
| | α-Amylase (activity) | β-Amylase (activity) | Lasche C T value | α-Amylase (activity) | β-Amylase (activity) | Lasche C T value |
| | *mg maltose* | *mg maltose* | *minutes* | *mg maltose* | *mg maltose* | *minutes* |
| Upon completion of germination | 51 | 785 | — | 52 | 788 | — |
| Final dried malt | 67 | 785 | 8.75 | 59 | 733 | 9.75 |

With this rapid drying procedure, activation of the enzyme was less pronounced. Nevertheless, the respective increases in alpha-amylase activity, from green to finished malt, were of the same magnitude as those shown in Table VI, when calculated as percentage gains.

## Summary

A pronounced increase in alpha-amylase activity was achieved by subjecting green barley malt to a "stewing" process in the top kiln (40°C [104°F] for 20 hours at a high moisture level) preceding final kilning. Beta-amylase activity was also enhanced, but to a lesser degree.

The gain in amylase activity during the controlled kilning resulted largely from an increase in alpha-amylase activity and hence differed from the gains brought about by an additional day of germination, which are due chiefly to increased beta-amylase activity. Moreover, a larger gain in alpha-amylase than is usually found by a seventh day of germination can be accomplished in the laboratory in from one to two hours.

A sulfur dioxide treatment, applied during kilning, appeared to stabilize amylolytic activity against heat inactivation during the final phases of kilning.

Modification of the kilning procedure can result in the production of malts with considerably enhanced amylase activities over those found when customary practice is followed.

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## BOOK REVIEW

**The Chemistry and Technology of Food and Food Products. II.** Prepared by a group of specialists under the editorship of Morris B. Jacobs. 890 pp. Interscience Publishers, Inc., New York, N. Y. 1944. Price $10.50.

Twenty-seven collaborators have contributed to the vast amount of information presented in this second volume of a two-volume survey of the chemistry and technology of foods. The contributions are divided into four major parts: Unit Operations and Processes (III), Sanitary and Quality Control (IV), Preservation (V), and Production (VI).

Part III comprises two chapters by Gaver describing the various types of equipment and methods used for such operations as storage, disintegration, clarification and concentration, mixing and coating, heat treatment, refrigeration, and dehydration. Specific examples illustrate the practical applications of such operations.

Part IV deals with the related topics: Food Supervision by Government Agencies (Trichter and Pincus), Food Grading (Samuelson), Food Machines (Abrahamson), Washing, Detergency, Sanitation, and Plant Housekeeping (Wilson), Insect Control: Insecticides and Fungicides (Shepard), and Prevention and Control of Rodent Life (Holsendorf). The two latter subjects are of considerable interest to the cereal technologist, but the brevity of the discussion presented will seriously limit the value of these chapters for practical purposes. They will serve mainly as a guide to the current literature cited in the bibliography.

Preservation is discussed from several points of view: The Dehydration of Foods (Mrak and Mackinney), Food Preservation by Temperature Control (Tressler), Preservation of Food in Hermetically Sealed Containers (Williams), Food Preservation by Use of Microorganisms (Fabian), Chemical Preservatives (Jacobs), and Packaging (Browne).

Technologists interested in dehydration will appreciate the references to the current problems in this field, particularly those related to palatability. The chapter on Freezing is rather brief and contains very few references to the literature. The chapters on Canning and Preservation by the Use of Microorganisms and Chemicals, while brief, are well-referenced and present salient features of these fields. Packaging is discussed rather inadequately from the standpoint of food preservation. A more

critical evaluation of existing data, together with a careful listing of references to the literature, would have materially enhanced the value of this chapter for many food technologists.

Approximately one half of the book is devoted to the production of a variety of foods: Cereal Grains (Geddes), Bread and Bakery Products (Cathcart), Sugars and Sirups (Jacobs), Confectionery and Cacao Products (Schoen), Fruit Juices, Jams, Jellies, and Preserves (Neubert and St. John), Milk and Milk Products (Corash), Meat and Meat Products (Urbain), Oils, Fats, and Related Products (Bailey), Nonalcoholic Beverages (Hale), and Alcoholic Beverages (Valaer). The final chapter considers problems of Industrial Waters (Nachod and Nordell).

The chapter dealing with Cereal Grains outlines in some detail the methods used in milling barley, corn, oats, rye, and wheat. Topics related to malting, bleaching, enrichment of flour, and the manufacture of macaroni and ready-to-serve breakfast cereals are also presented. The discussion is well-referenced, and the author has enhanced its value by including data obtained from authorities in the several industrial fields.

Bread and Bakery Products includes a description of the methods employed in the production of bread, rolls, crackers, cookies, cakes, and doughnuts. Numerous photographs of commercial bakery equipment in operation and descriptions of its use add to the clarity of the presentation.

Milk is discussed principally from the standpoint of liquid milk, together with products such as creamed butter, frozen desserts, cheese, and condensed milk. For information about dried milk, the reader is referred to the chapter on Dehydration. Turning to this reference, two short paragraphs are revealed! The cereal chemist seeking information about dried milk should choose some other source.

Another chapter of interest to the cereal technologist is Oils, Fats, and Related Products. Methods used for extracting, refining, and hardening of oils are described, together with the techniques employed for plasticizing and producing "high-ratio" shortenings.

The editor has pointed out that a collaborative effort such as that which made the present volume possible can scarcely avoid duplication. In many instances the duplication is most welcome, since it presents several viewpoints. On the other hand, attempts to avoid duplication seem to have resulted in failure to consider several important subjects, or in dealing with them only inadequately. It is hoped that an attempt to remedy these weaknesses will be made when revision is considered. Considering the times, when it must have been difficult to find authorities who could devote the necessary effort, and in view of the complexity of the field of food chemistry and technology, the editor and authors are to be congratulated on their attempt to fill an important void. Every food technologist who wants to know something about fields other than his own specialty will welcome this opportunity to have a condensed source of information.

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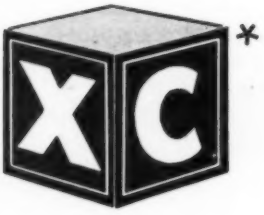


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